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The late Dr. H. P. Talbot was consulting editor of the series from its inception in 1911 until his death in 1927. Dr. James F. Norris was consulting editor from 1928 until his death in 1940. Dr. Louis P. Hammett became joint consulting editor in January, 1940.

QUANTITATIVE AGRICULTURAL ANALYSIS

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PREFACE

The time is, happily, past when "Chemistry for Medical Students," "Quantitative Analysis for Engineers" and similar titles, indicating treatises on the spoon-feeding of special dishes of easy chemical cookery to the classes of persons indicated, met any very general demand on the part of teachers. Even in our highly specialized chemical science of today and in its enormously diversified applications to industrial and economic problems, we recognize the futility of attempting to train students for technical or professional careers by teaching them only the mechanical notions and processes of chemistry without the scientific development of fundamentals.

The authors have tried to keep this idea in view in the compilation of this book. The discussion of special methods (largely "official," wherever applicable) for the analysis of materials of prime importance to chemical students of agricultural materials and of agricultural problems forms an important portion of the book; but we subscribe very heartily to the belief that one of the things most needed by scientific agriculture today is an increasing body of agricultural chemists who understand the importance of desiring to know *why* matters are thus and so.

The introductory course in general quantitative analysis, in Part I, deals with a select list of such analytical processes as may be considered useful for impressing upon the mind of the student the principles of analytical work, as well as the importance of exercising intelligence and care in all work of the laboratory. Bearing in mind the fact that in most college curricula this first course must necessarily be brief, the typical classes of methods for a given determination have been treated together. This, in turn, has involved a preliminary discussion of materials and methods of both gravimetric and volumetric analysis.

Part II, dealing with certain special measurements, has been included in recognition of the fact that the highly important instruments and methods there discussed are too seldom understood by the chemists who use them in industrial work. In our own classes we have found lectures upon the theoretical principles underlying the construction and use of these forms of apparatus to be of very great value.

In Part III is included a treatment of the six classes of materials most often considered in courses in agricultural analysis, and probably of interest to the greatest number of agricultural chemists. The significance of the results of the analyses, in connection with agricultural problems, has been given as much attention as was thought possible, without going outside the proper scope of a book of this character. This, it is believed, will add an interest to the laboratory work and supply a certain motivation, otherwise largely lacking.

In certain parts of the book we have drawn rather freely upon portions of another text by one of us.[1] This is particularly true in the discussion of materials and general operations, of the analysis of oils, fertilizers and dairy products and of the determination of nitrogen. Certain cuts have been borrowed from the same source, while others are from original drawings, made by G. B. Wilson.

Problems in analytical calculations have not been included. Several good problem texts are now available and the authors believe that a systematic course with one of these, as an accompaniment to the laboratory work and lectures, is the best method of impressing this phase of the subject upon the mind of the student.

E. G. Mahin,
R. H. Carr.

Purdue University,
September, 1922.

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[1] Mahin, "Quantitative Analysis."

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INTRODUCTION

For the most part the operations of analytical chemistry fall naturally into quantitative lines. This is particularly true of analysis as applied to agricultural problems because the qualitative composition of most agricultural materials is usually fairly accurately known from the nature and proposed use of the materials themselves.

The qualitative method for the detection of a given element or compound frequently involves the use of the same reactions as those that are fundamental to the quantitative determination of the same materials and in these cases, especially, it is most convenient to modify the details of the experiment so as to make a quantitative determination possible in the beginning, rather than to repeat the work in this manner after the completion of a qualitative analysis. This is not universally true and there will be occasional instances in which the complete qualitative analysis will save the labor of quantitative determination of elements not present in any significant quantity.

As the name implies, quantitative analysis has for its object the determination of the *quantity* (usually, though not always, expressed as per cent) of the various constituents of a material under investigation. The constituents determined may be elements or radicals of a compound, mixture or solution. The particular method to be used for a given material will be chosen according to circumstances and, to some extent, according to individual preference or available equipment. It will necessarily be modified if interfering substances are present. On this account it is desirable first to learn a few methods for the quantitative determination of some common elements in pure compounds and later to apply these and other methods to a more extended analysis of more complicated materials.

QUANTITATIVE AGRICULTURAL ANALYSIS

PART I

GENERAL ANALYSIS

CHAPTER I

THEORY AND GENERAL PRINCIPLES

Gravimetric Analysis.—When the quantitative composition of a material is learned through the direct application of the analytical balance the method is known as a "gravimetric" one. In principle the method is comparatively simple. A certain quantity of the well mixed sample is weighed accurately. It is then subjected to a series of operations, as a result of which a certain element or radical is finally separated from other constituents, either in its simple form or, as is more often the case, that of a pure compound of known formula. The latter is then weighed accurately. The two weights thus obtained and the known composition of the pure compound provide the necessary data for the calculations.

The determination of phosphorus in a phosphate rock may be taken as an example. The rock may contain ordinary tricalcium phosphate, $Ca_3(PO_4)_2$, as its chief constituent but it will also contain varying quantities of other materials, such as clay, quartz sand, limestone and iron oxide, so that the formula as given above cannot be assumed to be a correct representation of the composition of the material. The latter is therefore carefully sampled and a small portion is accurately weighed. It is then treated with an acid and the insoluble silica and silicates are removed by filtration. All of the phosphorus is then precipitated as ammonium phosphomolybdate, a complex substance represented by the formula $(NH_4)_3PO_4.12MoO_3$. This is filtered out, washed, redissolved and finally precipitated as magnesium ammonium phosphate, $MgNH_4PO_4$, which is washed and then changed to magnesium pyrophosphate, $Mg_2P_2O_7$, by heating strongly in a previously weighed crucible. From the weight of the crucible, with and without the pyrophosphate, the weight of the latter is found.

Factors.—The formula for magnesium pyrophosphate shows that it contains 27.87 per cent of phosphorus $\left(\frac{2P \times 100}{Mg_2P_2O_7} = 27.87\right)$. Multiplying this figure by the weight of pyrophosphate found and dividing the product by the weight of sample gives the per cent of phosphorus in the phosphate rock. Stated as a formula:

$$\frac{2 \times 31.04 \times 100\ W}{222.72\ S} = \text{per cent P in sample,} \qquad (1)$$

where W = grams of magnesium pyrophosphate found and S = grams of sample taken. No matter how many different samples of rock or other material might be subjected to this experimental process, the calculation would always follow the lines indicated in Eq. (1) and, since the only variables in this equation are the weights of sample and of pyrophosphate, the constants may be collected:

$$\frac{2 \times 31.04 \times 100}{222.72} = 27.87 = F. \qquad (2)$$

The quantity F is called a "gravimetric factor" and, since the procedure for phosphorus as already outlined is an illustration of the procedure for all gravimetric determinations, this factor may be calculated once for all for each type of determination and recorded, together with its logarithm, in a convenient place. Equation (1) is then a special application of the more general equation:

$$\frac{F\ W}{S} = x, \qquad (3)$$

F always indicating the per cent of the determined element or combination of elements in the *weighed precipitate*, as calculated from the chemical formula, and x representing the per cent of the same entity in the *sample* analyzed.

As indicated in the preceding paragraph, a combination of elements (as an oxide or radical) may be calculated. For example the factor for phosphorus pentoxide would be

$$\frac{100\ P_2O_5}{Mg_2P_2O_7} = \frac{14208}{222.72} = 63.79.$$

Factor Weights.—In Eq. (3) F is a constant for all determinations of the particular element or group of elements for which it has been calculated. It is possible to choose the weight of the sample taken so as to simplify the calculation of this equation. For instance, by taking a sample weight equal in grams to the value of the factor, $\frac{F}{S}=1$ and Eq. (3) becomes:

$$W=x. \qquad (4)$$

In such a case the weight of precipitate, expressed in grams or fractions, becomes per cent, or fractions, of the constituent determined.

A weight of sample equal in grams to the value of the factor is usually too large a quantity to be handled readily and a definite fraction of this weight (as 0.5, 0.2, 0.1, etc.) may be used instead. Any such weight is called a "factor weight," which may be defined as *a quantity equal in weight units to the value of the gravimetric factor, or to some simple fraction of this factor.*

Continuing the illustration given above, the factor weight of sample actually taken would be, for the sake of convenience, 0.6379 gm, in which case the per cent of phosphorus in the sample would be one hundred times the weight, in grams, of magnesium pyrophosphate found.

When a Factor Weight Should be Used.—In considering the actual practice of the operations with the balance it will be found that the manipulation of the sample to obtain any previously specified quantity requires considerable time, if the weighing is to be done accurately. One cannot judge quantities accurately by means of the eye and it becomes necessary to adjust the sample while it is on the balance pan, very carefully by removing or replacing very minute quantities. On the other hand, it is a comparatively simple matter to take approximately the required quantity and to weigh this accurately, using the figure thus found in later calculations. It may then easily be seen that all of the convenience and time-saving element that is involved in the calculations where factor weights (or, in fact, any other definitely prescribed weights) have been used, may be more than lost in the time and trouble required for adjusting the sample weight to this exact value.

For the reason just mentioned it is inadvisable to use factor weights *except in cases where relatively large amounts of sample* *may be used or where no great accuracy is required.* In such cases the sample weight may be accurately and quickly adjusted to the second or third decimal and the remaining uncertainty will be relatively insignificant. For example, if a 10-gm sample of soil is to be used for a nitrogen determination, an uncertainty of 1 mg in weighing will involve only 0.01 per cent of the total nitrogen found. But if a 0.5-gm sample of limestone were to be used for a determination of calcium, this same uncertainty would amount to 0.2 per cent.

Temperature Systems.—In nearly all scientific work the Centigrade system is used exclusively for indicating temperatures and in this book all temperatures mentioned are in Centigrade *unless otherwise designated.* In some instances the special agricultural analyst will have to use the Fahrenheit system in order to conform to established usage. When this is done in the following pages, the letter "F" will follow the figures indicating the temperature.

Volumetric Analysis.—The final determination of per cent by volumetric methods is not made by means of weighing a precipitate. The balance is generally used, as in gravimetric methods, for weighing the sample. The solution of the latter is then brought into definite reaction with another solution of an appropriate reagent (a **standard solution**) until the reaction is exactly completed. The concentration of the standard solution is accurately known as a result of a previous analysis (a **standardization**) and the volume required is measured accurately by means of a graduated burette. The product of the required volume of the standard solution and its concentration, giving the weight of the dissolved reacting material, serves as a measure of the determined constituent of the sample, just as the weight of the precipitate does in gravimetric analysis, the only difference in principle being the use of the weight of a *reacting* body instead of that of a *containing* body as a measure of the thing to be determined. With this exception the calculations will be similar to those of gravimetric analysis, a **titration** serving instead of a weighing.

As an illustration, the determination of sodium hydroxide in an impure sample may be cited. A weighed quantity of the material is dissolved and titrated by a standard solution of hydrochloric acid, a drop or two of an appropriate indicator, as methyl orange or methyl red, being added to show the end point of the reaction.

If V = cubic centimeters of standard solution required, C = concentration of standard solution (gm of HCl per cc), S = gm of sample used, Eq_{HCl} = equivalent weight (see page 7) of hydrochloric acid (36.468), and Eq_{NaOH} = equivalent weight of sodium hydroxide (40.008), then

$$V\ C = \text{gm HCl used,} \qquad (1)$$

$$\frac{V\ C\ Eq_{\mathrm{NaOH}}}{Eq_{\mathrm{HCl}}} = \text{gm NaOH in sample used,} \qquad (2)$$

$$\frac{100\ V\ C\ Eq_{\mathrm{NaOH}}}{S\ Eq_{\mathrm{HCl}}} = \text{per cent NaOH in sample.} \qquad (3)$$

Of course this derivation is based upon the assumption that sodium hydroxide is the only basic substance present in the sample.

As in gravimetric analysis it is convenient to collect all of the constants of the final expression. For all determinations of sodium hydroxide that are made by means of this particular standard solution of hydrochloric acid, V and S are the only variables. The quantity:

$$\frac{C\ Eq_{\mathrm{NaOH}}}{Eq_{\mathrm{HCl}}} = \frac{40.008\ C}{36.468}$$

may be called the "base factor" of the acid. This can then be simplified and recorded upon the label of the bottle. Let this be designated by F_B. Thereafter, so long as this solution is used for the determination of sodium hydroxide in other samples, the calculation of the results of titrations will be made by means of the equation:

$$\frac{100\ V\ F_B}{S} = \text{per cent NaOH.} \qquad (4)$$

If the same standard solution is to be used for the determination of any other base it will be necessary to recalculate the value for F_B for this substance and to use the new value in an equation similar to Eq. (4). If a new standard solution of a different concentration is prepared, or if the concentration of the original standard has changed, a new value for F_B is calculated.

Adjustment of Sample Weight.—The volumetric calculations already explained have been made upon the assumption that the sample weight was not adjusted to any particular value although it was, of course, accurately determined. In Eq. (4) F_B is a constant for this particular standard solution in this particular determination. Therefore if some care is exercised in adjusting the sample weight, S, so that it will bear some simple relation to F_B, the calculations will be materially simplified. For example, if S is made to equal 100 F_B, Eq. (4) will become:

$$V = \text{per cent NaOH}. \tag{5}$$

That is, each cubic centimeter of standard solution used in the titration represents a weight of sodium hydroxide which is 1 per cent of the sample weight, so that the burette reading becomes a percentage reading. From this the rule follows:

To make the burette reading a direct percentage reading, use a sample weight equal to 100 F_B.

In practice it often happens that such an adjustment calls for a too small weight of sample and it does not then provide for sufficient accuracy. Ten or one hundred times this weight is often taken, making 1 cc of standard solution indicate tenths or hundredths of 1 per cent.

Use of Aliquot Parts.—If the adjustment of sample weight must be made with a high degree of accuracy it may be that the extra time involved in the adjustment will not be compensated by time saved in calculations, in which case such adjustment will not be desirable. But if relatively large samples may be used for the analysis an error in weighing becomes of proportionately less importance and adjustment may be made more rapidly and less carefully. These considerations apply as in gravimetric analysis (page 4).

The use of large samples is rendered practicable by the use of the principle of aliquot parts. Some simple multiple of the required weight is taken and the solution is diluted to a definite volume in a volumetric flask and well mixed. A definite fraction of this solution is taken for the analysis and the proper factor to correct for this is used in the calculation of results. For example, if a degree of accuracy carried to the fourth decimal place is required in weighing 0.3943 gm for a single analysis, ten times this weight, or 3.943 gm may be weighed to only the third decimal place, the same number of significant figures being determined in the two cases. This sample may be weighed much more rapidly than the first. One solution of sample thus serves for several different titrations.

The principle of aliquot parts is of service also in the analysis of materials that are not homogeneous and that cannot be mixed readily, the larger quantity being more nearly representative than the smaller one and the mixing being accomplished after the weighed sample has been dissolved.

Normal System.—In case it is possible to apply a given standard solution to the titration of a number of different substances (as a standard acid for various bases or a standard base for various acids), there is a certain convenience to be derived from adjusting the concentration of the standard so as to make F_B equal to one-thousandth of the equivalent weight of the substance determined, or to some other simple fraction of the equivalent weight, as 0.002, 0.0001, 0.0005, etc.

The "equivalent weight" of any element or group of elements is the *number of weight units of this entity that is chemically equivalent to eight weight units of oxygen.* In the case of elements this is the *combining weight.* In all cases the equivalent weights compose a series of relative weights of the various chemical entities, chemically equivalent to each other in reacting power. From this definition it is obvious that if F_B is to be made equal in grams to one-thousandth of the equivalent weight of the substance determined (or 1 milligram-equivalent), 1 cc of the standard solution must *contain* 1 milligram-equivalent of the active constituent. A solution of this concentration is a normal solution and the following relations are consequences of the definitions discussed above:

(*a*) One cubic centimeter of any normal solution is equivalent to 1 milligram-equivalent of any substance.

(*b*) One cubic centimeter of any normal solution is equivalent to 1 cc of any other normal solution.

Normal solutions are too concentrated to allow a very high degree of accuracy in analytical work and it is more often desirable to use half-, fifth-, tenth- or even hundredth-normal solutions for accurate work. The relations existing between solutions of various normalities will be seen from relations (*a*) and (*b*) above.

Volumetric Factor Weights.—If the rule given on page 6 for making the burette reading a percentage reading is followed when using the normal system, the result is the **volumetric factor weight** of sample. This, of course, becomes one-tenth of the gram-equivalent of the element or compound determined in the sample.

Decimal System.—A further simplification may be made by adjusting the standard solution until each cubic centimeter is equivalent to a simple fraction of a gram of the substance to be titrated, instead of to a simple fraction of a gram-equivalent as in the normal system. One cubic centimeter of a standard iodine solution might then be equivalent to 0.005, 0.002, 0.001, etc., gm of sulphur. This results in a very much simplified calculation and a further saving of time is accomplished by using a sample weight which bears a simple relation to the equivalence of the standard. In the case just noted the sulphur sample might be used in portions of 0.5, 0.2 or 0.1 gm, or of ten times these weights. Then 1 cc of standard solution would indicate 1 per cent or 0.1 per cent of sulphur.

Such solutions as these are frequently made for technical work in industrial laboratories, where large quantities of standard solutions are required for the titration of a *single constituent* of a *large number of samples*. Mention may be made of the use of potassium permanganate or potassium dichromate solutions for the titration of iron in ores, of sodium thiosulphate for the titration of copper in ores or available chlorine in bleaching powder and of potassium ferrocyanide for the determination of zinc. In fact any standard solution may be made in this system *and it should be so made if its use is to be limited to the determination of one substance.*

Standardization.—Thus far we have dealt only with the calculation of the *results* of volumetric analysis, assuming that the standard solution was ready for use in the experiment. The determination of the exact concentration of the standard solution is called "standardization." The details of the experimental work will be treated later and will be mentioned here only so far as they may serve to illustrate the methods used in the calculations.

Standardization may be accomplished by one or more of four general methods:

Direct Weighing.—The active substance of the solution is accurately weighed and dissolved so as to make a definite volume of solution. The method is applicable to only such substances as may be obtained in a pure state or in a state of uniform and accurately known composition. Most of such materials are crystallized salts or acids, or soluble gases.

Weighing a Substance Produced by a Measured Volume of the Solution.—Sulphuric acid solution may be standardized by adding an excess of barium chloride to a measured volume of the solution. From the weight of barium sulphate found the weight of sulphuric acid may be calculated. Similarly hydrochloric acid solution may be standardized by adding silver nitrate to a known volume of solution and weighing the silver chloride produced.

Measuring the Volume of Solution Required to React with a Known Weight of a Substance of Known Purity.—An acid may be allowed to react with a pure carbonate and the required volume noted. Sodium thiosulphate may likewise be titrated against a weighed quantity of iodine or (indirectly) against a weighed quantity of arsenic trioxide.

Titration against Another Solution Which Has Already Been Standardized.—This method is very much used in the laboratory.

Primary Standards.—It will be noticed that in each of these cases there is some substance of known composition which is measured or weighed and the solution is somehow compared with this for standardization. This substance of known composition is called the "primary standard," whether it be the substance dissolved in the solution, something produced by the solution or something reacting with the solution.

The following examples will illustrate the methods of calculation in each of the cases discussed.

1. The method of calculation for the first method of standardization is self-evident. The normality is equal to the ratio of the number of grams dissolved in 1000 cc to the number of grams in 1000 cc of a normal solution. That is,

$$\text{normality} = \frac{\text{gm per 1000 cc}}{\text{equivalent weight}}.$$

2. A solution of hydrochloric acid was standardized by precipitating the chlorine from 40 cc as silver chloride. The weight of silver chloride found was 0.6327 gm. Required, the normality of the solution.

$$1 \text{ cc acid solution} \Bumpeq \frac{0.6327}{40} \text{ gm silver chloride.}$$

$$1 \text{ cc normal acid solution} \Bumpeq 0.1433 \text{ gm silver chloride.}$$

$$\text{Therefore normality} = \frac{0.6327}{40} \div 0.1433 = \frac{0.6327}{40 \times 0.1433} = 0.1107 \text{ N.}$$

To make the solution decinormal 1000 cc would be diluted to 1107 cc.

3. A similar solution was standardized by titration of pure sodium carbonate in presence of methyl orange, the following reaction being completed:

$$Na_2CO_3 + 2HCl \rightarrow 2NaCl + H_2CO_3.$$

It was found that 32.2 cc acid $\Bumpeq$ 0.1638 gm of the primary standard, sodium carbonate. Required the normality.

$$1 \text{ cc acid} \Bumpeq \frac{0.1638}{32.2} \text{ gm sodium carbonate and}$$

$$1 \text{ cc normal acid} \Bumpeq 0.053 \text{ gm sodium carbonate.}$$

$$\text{Therefore normality} = \frac{0.1638}{32.2 \times 0.053} = 0.9598 \text{ N.}$$

4. Another acid solution was standardized by titration against a measured volume of standard potassium hydroxide solution in presence of methyl orange according to the equation:

$$HCl + KOH \rightarrow KCl + H_2O.$$

One cubic centimeter of the primary standard contained 0.00468 gm of potassium hydroxide. It was found from the titration that 50 cc of potassium hydroxide solution $\Bumpeq$ 43.5 cc of hydrochloric acid solution.

The weight of potassium hydroxide in 50 cc of solution = 50×0.00468 gm. Since this weight was equivalent to 43.5 cc of acid, the potassium hydroxide equivalent to 1 cc acid = $\frac{50 \times 0.00468}{43.5}$ gm. The normality of the hydrochloric acid solution $= \frac{50 \times 0.00468}{43.5 \times 0.0561} = 0.095$ N.

In case the primary standard is a solution already standardized in the normal system the normalities of the solutions are inversely as the respective volumes that are equivalent to each other.

5. Thirty cubic centimeters of $\frac{N}{10}$ sodium thiosulphate solution is found by titration to be equivalent to 29.8 cc of iodine solution. The normality of the latter is required.

This is $\frac{30.0}{29.8} \cdot \frac{N}{10} = 1.007 \frac{N}{10}$.

If solutions are to be standardized in the decimal system the calculations involve nothing more than finding the weight of the substance in terms of which the standardization is to be expressed, equivalent to 1 cc of the solution which is being standardized, always using as the starting point the known weight of the primary standard.

In many cases the standardization is to be expressed in terms of the primary standard itself. For example, iodine solution is to be standardized against pure arsenic trioxide and expressed in terms of the same substance. Here we have the very simple method of weighing a suitable amount of arsenic trioxide, then dissolving and titrating by the iodine solution. Then

$$1 \text{ cc iodine solution} \approx \frac{\text{gm } As_2O_3.}{\text{cc I-solution}}.$$

Other familiar examples of this class of methods are the standardization of permanganate solutions against oxalates or against elementary iron or antimony for obtaining the weights of these elements equivalent to 1 cc of the solution.

The following example will serve to illustrate the first case just discussed:

6. A solution of potassium permanganate was standardized against sodium oxalate as follows: 2.5340 gm of sodium oxalate was dissolved and the solution was diluted to 1000 cc. Twenty-five-cubic centimeter portions were titrated and gave an average of 24.25 cc of potassium permanganate solution equivalent to the oxalate solution used. Required the weight of iron and of calcium equivalent to 1 cc of permanganate solution.

Twenty-five cubic centimeters of the oxalate solution contained 0.025×2.5340 gm and 1 cc of permanganate solution is equivalent to $\frac{0.025 \times 2.5340}{24.25}$ gm of sodium oxalate. This weight, multiplied by the ratio of the equivalent weight of iron or of calcium to that of sodium oxalate, will give the weights of these substances that are equivalent to 1 cc of the standard solution. Then

$$1 \text{ cc solution} \approx \frac{0.025 \times 2.5340 \times 55.88}{24.25 \times 67.005} = 0.00218 \text{ gm Fe}$$

or

$$\frac{0.025 \times 2.5340 \times 20.035}{24.25 \times 67.005} = 0.00078 \text{ gm Ca.}$$

Indicators.—Any substance that is used to show the end point of a definite reaction is an "indicator." The indicator may do this by a change of color in solution or by the appearance of a precipitate. In some cases the standard solution itself or the substance titrated may act as indicator. A familiar example of this is the oxidation of iron by potassium permanganate. As long as any ferrous iron is present the intensely colored permanganate is reduced to practically colorless manganese salts but the least drop of permanganate in excess colors the solution and indicates the complete oxidation of all iron present. In this case, as with other color changes and precipitations of inorganic compounds, the reaction at the end is definite and well understood.

"Neutrality" Indicators.—The indicators that are used to show neutrality points in reactions of acids and bases with each other are usually organic and their color changes are reversible as the point of neutrality is passed in either direction. The color change is due to a change in molecular structure which, in turn, is in equilibrium with hydrogen or hydroxyl ions present in the solution.

Hydrogen Ion Concentration.—The volumetric titration of acids with bases, or conversely, is a process of neutralization. This is the production of a condition where neither hydrogen nor hydroxyl ions are present in more than very slight and negligible excess. Neither of these ions can be absolutely eliminated from any aqueous solution. Both must be present and in such proportion that the *product* of their concentrations is a constant, 10^{-14} gram-ions per liter. This product is a very small quantity and it is obvious that an acid solution (essentially a hydrion solution) must contain extremely minute quantities of hydroxylion, while basic solutions contain considerable concentrations of hydroxylion and correspondingly little of hydrion. At the "neutral point" the ion concentrations are equal, so that each of these two ions is present to the extent of 10^{-7} ($=\sqrt{10^{-14}}$) gram-ions per liter. This is the relation for pure water also and it is expressed as follows:

$$[H^+]=[OH^-]=10^{-7}, \tag{1}$$

$$[H^+]\times[OH^-]=K_W=10^{-14}. \tag{2}$$

Since Eq. (2) expresses a condition existing in all aqueous solutions of electrolytes, it will be seen that the concentrations of the essential ions of acids and bases cannot be independent but that they must vary inversely, so that both "acid" and "basic" conditions might be represented in terms of either one of these ions. Following the suggestion of Sorensen the expression $-\log\ [H^+]$ is used for this purpose and the symbol $P_H{}^+$ is used to indicate this quantity. This symbol has been variously modified to pH or P_H. So long as ion concentrations are expressed as powers of 10, as above, P_H will be the same as the negative exponents of 10. Reference to Eq. (1) shows that the neutral condition will be expressed by the statement

$$P_H = 7 \text{ (strictly, 7.03 at 20°)}. \tag{3}$$

For acid solutions P_H *is always less than* 7 *and for basic solutions it is always greater than* 7.

It has already been remarked that indicators are themselves acids or bases, as in solution they yield hydrion or hydroxylion, or both (amphoteric indicators) and the concentrations of these ions are definitely related to the equilibrium concentrations of the tautomeric forms of the indicator, finally responsible for the color changes. Therefore the color changes of the indicator will follow the change in the value of P_H as neutralization of the solution is approached.

The use of indicators for the determination of actual hydrogen ion concentration has been highly developed and this use finds a wide application in many fields of applied chemistry. This phase of the subject is briefly treated on pages 138 to 140. However, we should note here that in making titrations in analytical chemistry we are usually not concerned with *existing ion concentrations*, but rather with total *ionizable* or *titratable* hydrogen or hydroxyl. In other words, we seek complete calculated neutrality, with chemically equivalent quantities of titrated and titrating substances present, rather than what might be called electrolytic neutrality, where P_H equals 7.

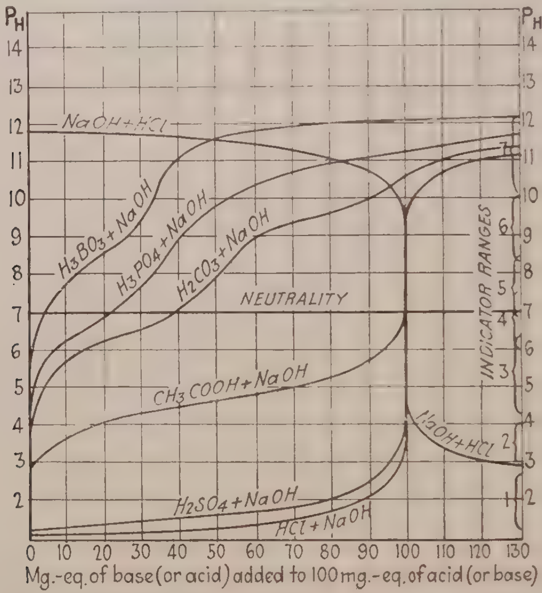


FIG. 1.—Typical titration curves. Indicator ranges are: 1-Thymol blue; 2-Methyl orange; 3-Methyl red; 4-Brom thymol blue; 5-Phenol red; 6-Phenolphthalein; 7-Alizarin yellow.

The curves shown in Fig. 1 illustrate the variation of P_H values as titration proceeds, in a few typical cases. As the titrating standard is added to a definite quantity of the titrated substance, the value for P_H changes with regularity. Two essential conditions must obtain, in order that a sharp end point may be observed. These are (*a*) that the neutralization curve must show a steep inclination at the point of equivalent neutrality (*i.e.*, that a very slight increase in the amount of standard solution added shall produce a relatively large change in hydrion concentration at this point) and (*b*) that the range of P_H over which the indicator changes color must include some portion of this steep part of the curve and no other portion. The curves shown in the figure for the "strong" acids conform to condition (*a*).

In the curve for the neutralization of carbonic acid there is a comparatively sudden break near the point of half neutralization. This corresponds to the formation of sodium bicarbonate:

$$H_2CO_3 + NaOH \rightarrow NaHCO_3 + H_2O.$$

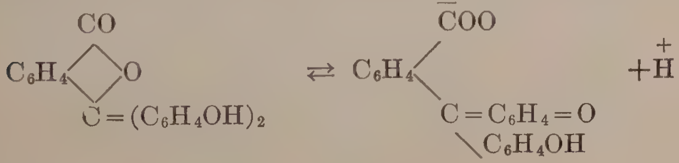
Any indicator whose color change covers only this portion of the curve will make a titration possible. Phenolphthalein, with a P_H range of 8.3 to 10, will serve for this purpose if the first appearance of pink (at the P_H value of 8.3) is taken as the end point. This point is, of course, not as sharp as could be desired. No indicator can be found that will give a sharp change with equivalent quantities of carbonic acid and a base, because of the gradual slope of the neutralization curve at this point.

Examination of the curves for boric and phosphoric (weakly ionized, polybasic) acids will show why these acids cannot be accurately titrated. The curve for boric acid shows a faint inflection at the point representing neutralization of the first of the three hydrogen atoms, but this would involve only a very gradual change in color of any indicator that would cover this range of P_H values. The case is quite similar for phosphoric acid.

Only a few of the common indicators are necessary for ordinary titrations in analytical work and three of these will be described briefly.

Phenolphthalein.—This compound is a white crystalline powder, almost insoluble in water but soluble in alcohol. For use in volumetric analysis a solution of 5 gm in 1000 cc of 50-per cent alcohol is suitable. One drop of this solution is sufficient for 100 cc of solution being titrated. The range of color change is P_H = 8.3 to 10.0.

Phenolphthalein is a derivative of phthalic anhydride and the solution contains two forms in equilibrium:



The second form predominates in basic solutions and the group $[=C_6H_4=]$ is in some way responsible for the red color. The first form is colorless and predominates in acid solutions.

Methyl Red.—This dye is *p*-dimethylaminoazobenzene-*o*-carboxylic acid:

$$(CH_3)_2N-C_6H_4-N=N-C_6H_4CO_2H.$$

The indicator solution is prepared by dissolving 1 gm of the solid in 100 cc of 95-per cent alcohol. The solution is pale yellow in basic solutions and violet red with acids. It is especially good for the titration of ammonium hydroxide and the alkaloids, all being weak bases. It cannot be used if much carbonic acid is present, hence is useless for the titration of carbonates. The color range includes $P_H = 4.4$ to 6.0.

Methyl Orange.—The methyl orange of commerce is the sodium salt of a sulphonic acid:

$$(CH_3)_2N-C_6H_4-N=N-C_6H_4SO_3Na.$$

This is a yellow substance which forms a yellow solution in water. In presence of acids the salt is decomposed and a red form, previously existing in equilibrium, now predominates. The color range includes $P_H = 2.9$ to 4.0.

A water solution containing 0.5 gm in 1000 cc is used as indicator in volumetric analysis. A single drop is usually sufficient to give a perceptible color to 1000 cc of solution.

The three indicators described above practically cover the range of hydrogen ion exponents from 2.9 to 10, with the exception of a gap between 6.0 and 8.3. This fact makes unnecessary the employment of indicators other than these three for the great majority of volumetric analyses, even when quite weakly ionized acids or bases are being titrated. It is nearly always possible to choose a strong electrolyte for the standard solution and one of these indicators will then generally serve to cover the portion of the curve that represents equivalent neutrality. The number of indicators that have been proposed and used for analytical purposes is very large. Many of these are useful for the determination of existing hydrogen ion concentration and these will be mentioned in a later chapter (page 139).[1]

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[1] For an exhaustive discussion of the whole subject of indicators see PRIDEAUX: "The Theory and Use of Indicators."

CHAPTER II

GENERAL OPERATIONS

Preparation of Samples.—The object of all preliminary work with samples is to make it possible to obtain, for the actual analysis, a portion that shall truly represent the average composition of the entire material at hand. This matter is likely to be treated lightly by the beginner, but proper sampling is often one of the most difficult problems of quantitative analysis. It is often necessary to use a quantity of 1 gm or less and if the substance is not homogeneous this small quantity may have an average composition that is very different from the average composition of the entire material being investigated. No matter how carefully an analysis may be performed or how accurate the results obtained, if the substance used does not represent the average of the substance originally at hand the results become nearly or entirely valueless. If the substance is practically homogeneous the operation of sampling involves nothing more difficult than grinding down to a degree of fineness required for the work. This is the case when the substance is an approximately pure chemical compound, such as will be used for the earlier exercises.

The gross sample, as the analyst receives it, may be in the form of lumps, as is frequently the case with minerals, or it may be in the form of small pieces, crystals, powder, or solution. In any case except that of liquid samples, the object is to reduce the size of pieces to that required for the analysis (usually a rather fine powder) and at the same time to select from the total mass such a quantity as is required for the experimental work. The original sample is often quite large. It is obviously unnecessary and practically impossible to grind the entire amount into a fine powder. The operation then resolves itself into a thorough mixing and progressive grinding and dividing. Many forms of both hand and power grinders are in common use. For the first exercises nothing more complicated than a porcelain mortar and pestle will be required.

Mixing and Dividing.—The mixing and dividing is best carried out by using a sheet of oilcloth or paper and a spatula. In many laboratories it is customary to use oilcloth, particularly for mixing minerals. This is convenient but offers the possibility of contamination ("salting") of one sample by the remnant of one that has preceded it. It is better to use a large sheet of tough, flexible paper, which can be discarded after using. The sample, after having been broken down to the proper maximum size of pieces, is placed on the paper and thoroughly mixed by rolling diagonally across the paper and alternating the direction of rolling as illustrated in Fig. 2. The proper rapid manipulation of the paper is attained only after considerable practice. One precaution is essential: the corner of the paper that is lifted must be drawn across, *low down*, in such a manner that the pile of material is not caused to slide along the paper but is turned over upon itself so that the bottom is brought entirely to the top. In this way only can a segregation of larger and smaller particles be prevented. Since the larger and smaller particles usually have different composition it is essential that they should be thoroughly mixed. The number of times that the sample is rolled before dividing will depend upon the degree of homogeneity and the accuracy required in the analysis. In the assaying of gold and silver ores it is not unusual to require one hundred times.

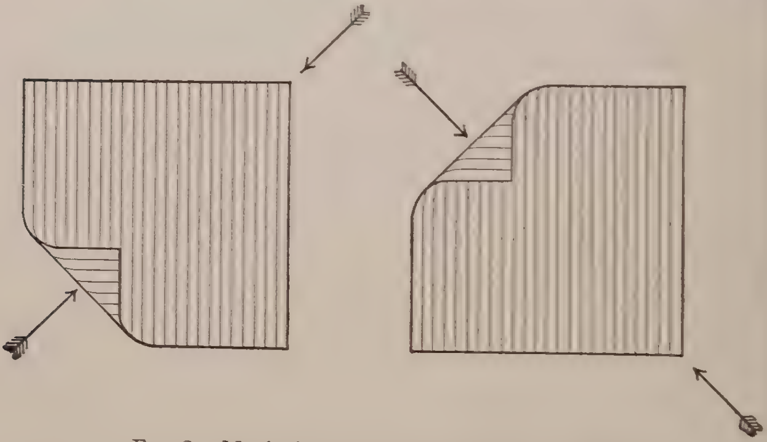

Fig. 2.—Manipulation of paper for mixing samples.

Quartering.—When the first mixing is finished the pile is made approximately circular and it is then divided, by means of a spatula, into quarters. *Opposite* quarters are carefully scraped to another sheet of paper, ground finer if necessary, remixed and quartered as before. This process of grinding, rolling, and quartering is continued until a sample is finally obtained, small enough in quantity and fine enough in texture to serve the purpose of the final weighing and analysis.

Maximum Size of Particles.—The maximum size of particles to be allowed in any particular mixing and quartering will depend upon the total quantity of material being handled in this operation. No particle should be so large that its inclusion in any quarter would cause the average composition of this quarter to be appreciably different from the average composition of the entire pile. This means that the ratio of the size of the largest particle to the size of the quarter should not be greater than a certain maximum value. What this maximum value shall be must be arbitrarily determined by the nature of the sample and the degree of accuracy required in the analysis. It is obvious that the part can perfectly represent, in composition, the whole only when the largest particle is infinitesimal. It is equally obvious that this limit is impossible and unnecessary in practice and we may say that, in general, the ratio of the largest particle to the portion that includes it should not be greater than 0.01 per cent. If this condition is met, then, after thorough mixing of the sample, the chance inclusion or exclusion of any given particle cannot modify the results of the analysis to any appreciable extent.

Other Considerations.—The maximum size of the particles to be obtained in the final portion that is to be weighed and used in the analysis must be determined, not only from the above considerations, but also by the nature of the operation to follow the weighing. This is usually solution or fusion. If the substance is considered to be almost absolutely homogeneous and if it is easily soluble (as, for example, a crystal of cupric sulphate) then the grinding need be carried no farther than is necessary to permit the easy adjustment, between fairly narrow limits, of the weight taken for analysis. In such a case, if a sample of 0.3 to 0.5 gm is required, then no particle should weigh more than about 0.1 gm. If, however, the process of solution or fusion is a difficult one to accomplish or if the material is far from being homogeneous, the grinding is carried much farther, in order to provide a very large surface of contact between the particle and the solution or flux, or in order to conform to the rule of maximum size of particles, stated above. In many cases, as with minerals, the maximum size of particles is fixed by causing the sample to pass through a sieve having meshes of stated dimensions. A gold ore may be ground to pass a sieve having 100 or 200 meshes to the linear inch. In such a case one should not make the mistake of grinding and sifting a portion until a sufficient quantity is passed, discarding the remainder. This would cause an error because the particles that resist grinding longest are less brittle and have a composition different from that of the particles which pulverize easily.

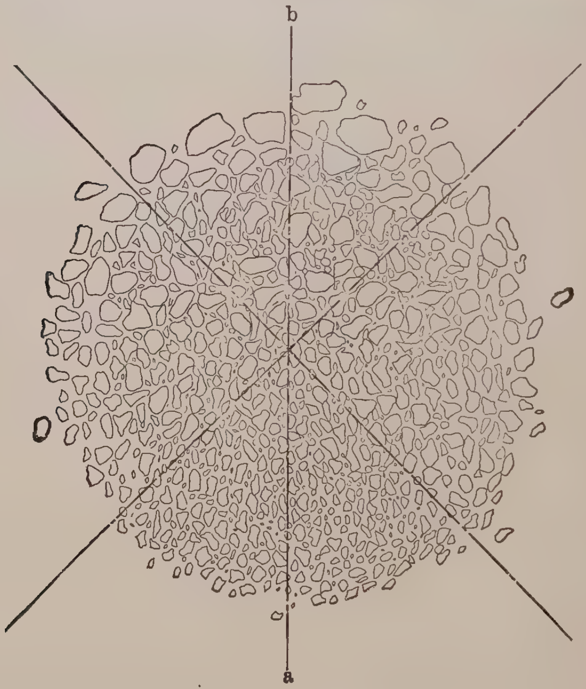

Fig. 3.—Division by quartering.

Effect of Quartering.—The reason for dividing into quarters after each mixing and for selecting *opposite* quarters will be understood from the following: Close examination of the pile of unmixed material will reveal the fact that, even after the most thorough and careful mixing, it is not entirely homogeneous. Around the circumference of the base the particles are coarser and they may be gathered toward one side. Around the apex of the conical pile there is a collection of coarser particles. If we simply dig in at random for the portion to be removed the lack of homogeneity will alter the character of this portion.

Figure 3 shows how the opposite quarters, no matter in what direction the cuts be made, will obtain the average of a non-homogeneous pile, while a cut into halves will do so only in case the cut is made in the direction *ab*. In these diagrams the conditions are purposely exaggerated.

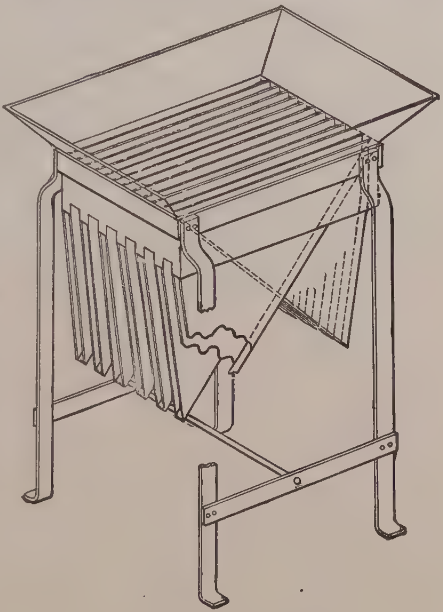

FIG. 4.—The riffle.

The Riffle.—Various forms of semi-automatic sampling devices are in use, designed to carry out the mixing and dividing process without laborious hand work. The riffle is one of these. As shown in Fig. 4 this consists of a hopper, at the bottom of which are placed several narrow chutes, so arranged as to transfer alternating adjacent portions of the crushed material to opposite sides and into separate pans (not shown in the illustration). This will have approximately the same effect as would cutting the pile of material into vertical, narrow sections, alternate portions being united so that the pile is finally halved. The riffle may be made of any convenient size, to handle large or small samples.

Sampling of Liquids.—In case the substance to be analyzed is a liquid the operation of sampling is usually a simple one, consisting of thorough mixing before the removal of the proper quantity for analysis.

Dissolving the Sample.—After the sample of substance has been properly selected and weighed the next operation is usually one of solution. What the solvent shall be is determined by the nature of the sample and by the character of the operations subsequently to be performed. Water may be used, or concentrated or dilute solutions of acids, bases or salts, organic solvents or solid substances used as fluxes by heating to high temperatures. In case gravimetric methods are to be employed it is desirable to use a relatively small quantity of the solvent, not only because it must finally be entirely removed, but also because all precipitates dissolve to some extent and it is only by keeping the amount of solvent down to the least quantity that is workable that the loss of precipitate is reduced to the minimum.

Fusion.—For the purpose of quantitative analysis the fusion of materials is almost always accomplished with the end in view of producing more soluble substances through the interaction of an added agent, called a flux, and the refractory material. For instance, most of the natural silicates are practically insoluble in water and all ordinary reagents and therefore they cannot be analyzed by ordinary methods. By a preliminary heating to a high temperature in contact with a basic substance like sodium carbonate, a fusible mixture of new compounds is formed and these will, for the most part, be soluble in water and hydrochloric acid so that the solution may be submitted to precipitation and filtration processes for the separation and determination of the elements. Similarly, refractory and insoluble metallic oxides may be heated with sodium pyrosulphate with the formation of a fused mass consisting of soluble sulphates of the metals.

The necessary qualities of any useful flux are (1) that it must be of such a nature as to be capable of reacting with the refractory body when heated with it and (2) that the resulting compounds shall fuse at the prevailing temperature. To these the analyst adds a third requisite: (3) that the resulting compounds shall be soluble in water or in the laboratory reagents. The first condition is met by choosing as the flux a substance of opposite nature to that of the refractory sample. That is, if the latter is of an acid nature (as silica and polysilicates) the flux should be basic, and conversely.

Fusibility.—No general statement can be made with regard to the relative fusibility of various compounds, as based upon the chemical composition of these compounds. It may be noted that refractory silicates are usually made more readily fusible by reducing the ratio of silica to metal oxide through the introduction of more metals, particularly of the alkali metals. Both of these points are made by using alkali metal carbonates as fluxes, since the net result of the reaction at high temperatures is to expel carbon dioxide and to combine the alkali metal oxide with the refractory silicate. This will explain why these carbonates are almost always chosen as fluxes for silicates. A reaction such as the following may occur when orthoclase is fused with sodium carbonate:

$$2KAlSi_3O_8+6Na_2CO_3 \rightarrow K_2SiO_3+5Na_2SiO_3+2NaAlO_2+6CO_2,$$

a more or less complicated mixture of aluminates and silicates of the alkali metals being formed.

Basic Fluxes.—Sodium carbonate, potassium carbonate and sodium-potassium carbonate are the most important of the basic fluxes that are used for analytical purposes. These are used chiefly for fusion with silica and the refractory silicates. Such fluxes as calcium oxide, used for fluxing silicates in the blast furnace for iron, are of little use for analytical purposes, partly because the resulting compounds are not soluble and partly because metals that are to be determined in the sample are introduced by the use of such materials.

Acid Fluxes.—Fluxes of an acid nature are valuable chiefly for forming fusible, soluble compounds when heated with metallic oxides or salts that are over-saturated with metallic oxides. The most useful of such fluxes are the pyrosulphates and the biborates of sodium and potassium.

Acid sulphates are often used instead of pyrosulphates. When the former are heated they give off water and they are completely converted into pyrosulphates by heating to higher temperatures:

$$2NaHSO_4 \rightarrow Na_2S_2O_7 + H_2O.$$

Because of the excess of sulphur trioxide in the pyrosulphate, this readily reacts with metallic oxides when heated with the latter:

$$Fe_2O_3 + 3Na_2S_2O_7 \rightarrow 3Na_2SO_4 + Fe_2(SO_4)_3.$$

The biborates likewise combine with metallic oxides because of their excess of boric anhydride.

$$Fe_2O_3 + 3Na_2B_4O_7 \rightarrow 2Fe(BO_2)_3 + 6NaBO_2.$$

Precipitation.—The process of precipitation is usually a chemical reaction between substances in solution, the result being the production of another substance of relatively small solubility. The actual precipitation is always preceded by a condition of supersaturation (with respect to the precipitating substance) and this breaks down at different rates with different precipitates. In some cases equilibrium between the precipitate and the saturated solution of the same substance is attained only after the lapse of considerable time, while in other cases such equilibrium results very quickly. An example of the first class of precipitates is found in magnesium ammonium phosphate. In order to obtain the greatest possible amount by precipitation the solution must be allowed to stand for some hours in contact with the crystals that have already been precipitated. Stirring usually serves to hasten the attainment of equilibrium by promoting contact of solid with the supersaturated solution.

It is important to note that there is no definite relation between the degree of *persistence of supersaturation* and the degree of *solubility at final equilibrium.*

Solubility Product.—In order to diminish the solubility of the precipitate to the lowest possible figure, use is made of the principle that in any saturated solution the product of the concentrations of the constituent ions is a constant (the "solubility product") which cannot be exceeded without reproducing the abnormal condition of supersaturation. By adding an excess of the precipitating reagent (and therefore of one of the ions of the substance precipitating) the concentration of this ion is largely increased. There must then be a corresponding decrease in the concentration of the ion that is being determined and this results from increased precipitation.

Colloidal precipitates, such as aluminium hydroxide, manganese sulphide, etc., do not obey the law of solubilities referred to above.

Size of Crystals of Precipitates.—Other conditions being unchanged, it may be said in general that slow precipitation results in the formation of relatively large crystals and conversely. The rule that the precipitating reagent should be added slowly and with continued stirring is a consequence of this fact. But if it has been found impossible to produce a precipitate of sufficient coarseness to permit retention by the filter paper this fault may usually be remedied by warming the solution and precipitate for some time. The actual result is the resolution of small crystals and the reprecipitation of their substance upon the larger ones. This is due to the fact that very small particles have a slightly greater solubility than larger ones. The process of heating a solution with its precipitate in this manner is called "digestion."

Filtration.—After a precipitate has been separated by filtration and washed, it is either dried to constant weight or strongly heated ("ignited") in a crucible, in order to bring about some definite change in its composition before weighing. In the former case it is practically necessary to use a filter of inorganic material because paper cannot be dried to any constant degree of hydration. If strong ignition is to be employed, either paper or inorganic materials may be used unless burning organic matter exerts a reducing action upon the precipitate, in which case the use of paper filters is again excluded.

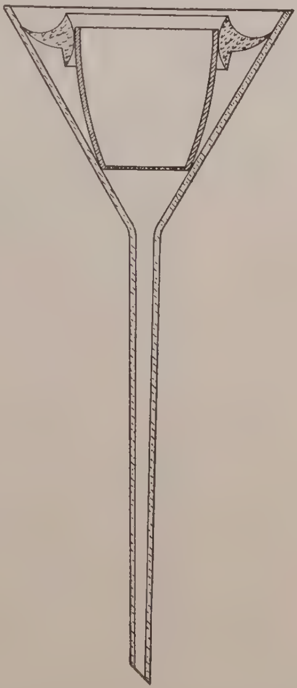

Fig. 5.—Gooch or alundum crucible with rubber filtering ring in funnel. (In section.)

Filter Paper.—For quantitative purposes a paper of very high grade is required. The texture must be close and uniform and the material as free as possible from inorganic matter which would be left as an ash on burning. To obtain the latter condition, paper is subjected to a preliminary extraction with hydrochloric and hydrofluoric acids, thus dissolving all but a small trace of ordinary ash-forming matter. Such paper is usually called "ashless."

Inorganic Filters.—To avoid the reducing action of the filter either an alundum crucible or a Caldwell crucible may be used. Alundum is a porous form of aluminium oxide, partly fused together with a binder. A crucible of this material may be placed in a rubber holder placed in a funnel, as shown in Fig. 5, and the liquid drawn through by suction. The precipitate is then washed and ignited directly in the crucible.

The Caldwell crucible (usually known as a "Gooch") is a tall crucible of porcelain whose bottom is perforated by small holes. This is used in a manner similar to that described for the alundum crucible, with the exception that a pad of asbestos (see page 158) is formed over the bottom and this provides the necessary filtering surface. For high temperatures the platinum form is better. This is the original Gooch crucible.

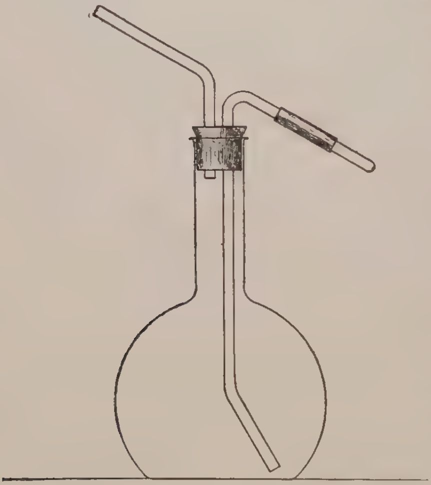

Fig. 6.—Common form of wash bottle.

Washing.—Wash bottles like Fig. 6 should be provided. A fine stream of water, hot or cold, may be blown on to the filter, the precipitate and filter being thus washed free from soluble impurities. To avoid unpleasant effects due to blowing back of steam from the hot water bottle, or of volatile liquids when these are used for washing in special cases, a pressure bulb and vent may be provided.

Great care must be used to avoid mechanical loss of precipitate. It is well to remember also that the most efficient washing is accomplished by using several small portions of wash water, rather than fewer and larger portions.

Drying.—If a precipitate is to be subjected to strong ignition it is not usually necessary to carry out any preliminary drying, other than such as may be performed in the crucible over a low flame. But some precipitates are to be weighed, after drying at a definite temperature, in which cases a drying oven having a fairly close temperature regulation must be provided. For this purpose the use of electrically heated ovens having automatic temperature regulation is now almost universal. Any oven must have provision for continuous displacement of humidified air by drier air. Passing the entering air through a drying agent, such as calcium chloride or sulphuric acid, will facilitate the drying operation but this is done only in special cases.

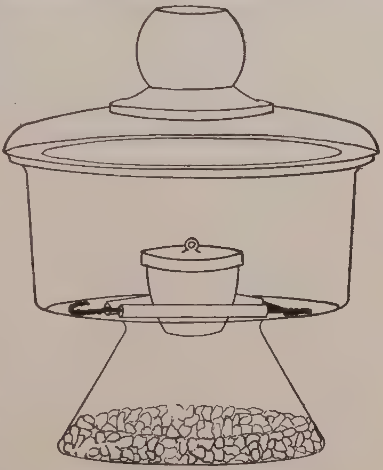
Fig. 7.—Small desiccator.

In order to understand the principle of drying it is well to recall the law that any moist substance will continue to lose moisture by evaporation until a certain definite pressure of water vapor ("aqueous tension") is established in the surrounding space, the value of this pressure depending upon (*a*) the nature of the moist substance and (*b*) the temperature. If the pressure of the surrounding vapor is reduced by extraneous means, evaporation proceeds until equilibrium is again established. Thus by continuing to reduce the external vapor pressure, evaporation may be continued. However, it is important to note that the vapor pressure to be considered is not the total pressure (such as that of the atmosphere) but the partial pressure of the vapor of water. As the latter pressure is directly proportional to the *concentration* of water vapor in the surrounding space, the same result will finally be produced (*a*) by reducing the total pressure by means of a pump, (*b*) by continuously displacing the moist air by means of some other dried gas or (*c*) by confining the moist substance in a space which contains some hygroscopic material.

Certain types of ovens make use of methods (*a*) or (*b*), above. In such ovens an air-tight chamber is provided and a dried gas is passed through this or the chamber is exhausted by means of an air pump.

Desiccators.—Method (*c*) is employed in the various types of desiccators, used at ordinary temperatures. Figure 7 illustrates a small desiccator suitable for carrying about the laboratory. In Fig. 8 is shown a desiccator in which are used the principles of methods (*a*) and (*c*). This is what is known as a "vacuum desiccator." In both illustrated forms of apparatus the drying agent, which may be calcium chloride, sulphuric acid, or, in certain special cases, phosphorus pentoxide, is placed in a layer on the bottom.

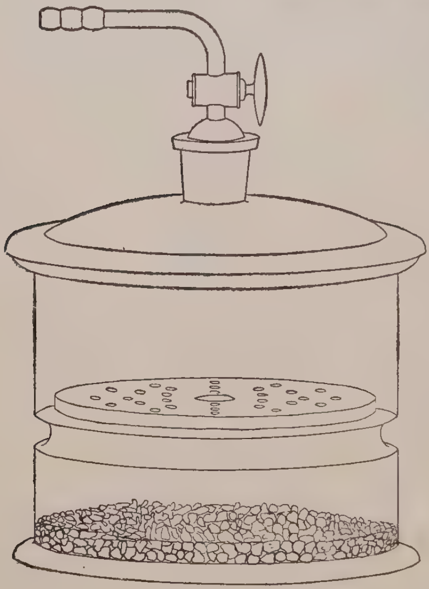

FIG. 8.—"Vacuum" desiccator.

Ignition of Precipitates. The term "ignition" is used in this connection in a sense somewhat beyond its ordinarily accepted meaning, since it is applied to the heating to high temperatures of substances that are entirely incombustible. The purposes of ignition are to destroy the filter, if paper has been used, to expel the last traces of moisture and volatile impurities that have not been removed by washing and to cause the precipitate to change in a definite manner, if a change is to be made. If a paper filter has been used it is carefully removed from the funnel by slipping up the side. It is then folded as indicated in Fig. 9, the object being so to enclose the precipitate that loss is impossible. If it is to be dried and removed it is then placed in the oven on a cover glass.

Oxidation in the Crucible.—The crucible is almost invariably heated by means of a naked flame, being supported on a triangle by means of some kind of stand. When the object is to oxidize the paper or precipitate the crucible is placed on its side and the cover leaned against it as shown in Fig. 10. The burner is placed under the bottom of the crucible in such a position that the gaseous products of the burner cannot enter the crucible. The uprising current of warm air strikes the cover and is deflected into the crucible, thus providing an oxidizing atmosphere about the paper. If the flame from the burner is applied only enough to keep the paper burning the desired condition is attained. No harm results if the volatilized combustible material from the paper burns with a flame above the crucible. After the paper is thoroughly charred the temperature is gradually raised to complete the combustion.

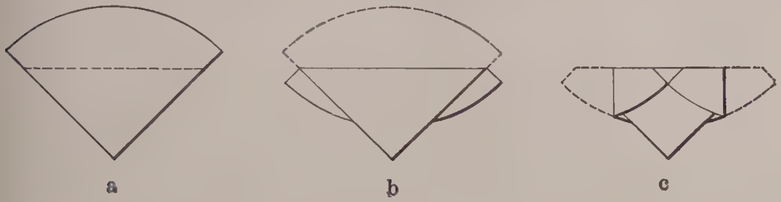

Fig. 9.—Method of folding a filter paper for ignition.

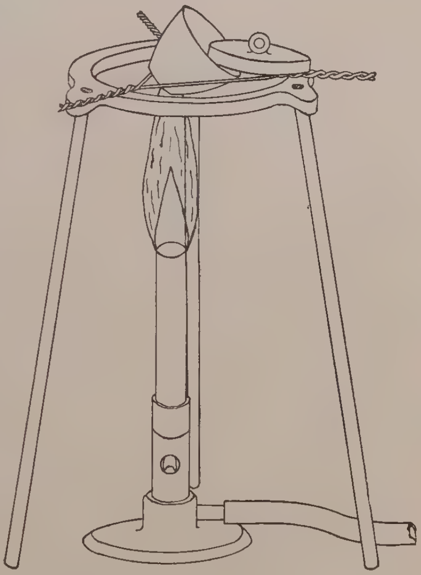

Fig. 10.—Position of crucible over flame for oxidation.

The proper position of the crucible on the triangle is shown in Fig. 11. If placed as in Fig. 12 the crucible is liable to fall back and it may even sometimes fall through and cause a failure of the determination.

Even in cases where the burning paper has no reducing action upon the precipitate it is still desirable to complete the combustion of the paper at a comparatively low temperature. *This is a matter that is too often ignored by the student.* Crystalline precipitates that are ordinarily regarded as infusible will often undergo softening at the sharp corners of the crystals. This causes a certain sticking together which results in the enclosure of a small amount of carbon in such a way as to make its oxidation extremely difficult. If the paper containing the precipitate is heated to a high temperature at the very beginning it is often almost impossible to make it white. One of the best examples of this action is in the ignition of magnesium ammonium phosphate to convert it into magnesium pyrophosphate. Premature heating of this substance to very high temperatures will frequently result in a black or gray material that cannot be whitened by long ignition.

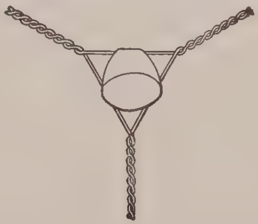

Fig. 11.—Correct position of crucible for oxidation.

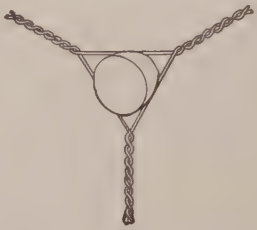

Fig. 12.—Incorrect position of crucible for oxidation.

Decomposition in the Crucible.—After oxidation of the paper is completed the temperature is raised in order to volatilize completely any volatile impurities that may remain and to cause whatever decomposition is desired. Since oxidation is no longer an object the crucible is placed in an upright position and the cover is placed over the top. This gives an opportunity for the flame to bear directly on the bottom of the crucible where the precipitate lies. The cover also largely prevents loss of heat due to convection currents of air within the crucible.

Crucibles.—Porcelain crucibles of high grade may be used for most work, in cases where the precipitate is not to be fused. Alundum already has been mentioned in connection with filtering crucibles. When any compound or mixture is to be fused, porcelain is usually unsuitable because the fused material will combine with the glaze, or even with the porcelain itself. For such work platinum or one of the newer substitutes, palau or rhotanium, is essential.

Marking Crucibles.—Metal crucibles should have permanent identification numbers stamped upon them by means of small dies. These numbers form a part of the analytical record and they serve to prevent accidental transposition of weight records.

Porcelain crucibles may best be marked by means of a pen and ink, the marks being inconspicuous figures or small dots. When the crucible is strongly heated the iron of the ink forms the red oxide, which burns into the glaze and forms a permanent identification mark. (Certain common inks do not contain iron, so that they are unsuitable for this purpose.)

Some manufacturers of chemical porcelain now furnish crucibles and dishes serially numbered with permanent marks. This is a great convenience to the analyst.

Care of Platinum.—Platinum ware will deteriorate rapidly unless the following precautions are taken in its use and care.

1. Handle carefully to avoid bending. Use clean crucible tongs and do not allow the tongs to come into contact with fused materials within the crucibles or dishes.

2. For cleaning apply the appropriate solvent, according to the nature of the material to be removed. Chromic acid is suitable for removing organic matter, and hydrochloric or nitric acids for insoluble carbonates or metallic oxides; fusing with sodium carbonate is suitable for removing silica or silicates, or with sodium pyrosulphate for such metals or metallic oxides as resist the action of acids.

3. Do not heat platinum in contact with the inner cone of the laboratory burner, as brittleness results from such exposure.

4. Do not heat compounds of lead, tin, bismuth, arsenic, antimony or zinc in contact with platinum. Reduction may occur, the reduced metal alloying with the platinum.

5. Do not attempt to remove fusions from platinum crucibles or dishes by means of files, glass rods or other hard tools. Use solvents or a rubber-tipped rod.

6. Dull surfaces should be polished lightly with wet emery slime or fine carborundum.

Platinum Substitutes.—The increasing scarcity of platinum has made the introduction of substitutes a practical necessity. While it is true that pure platinum possesses certain properties that cannot be duplicated by any other metal or alloy, yet certain alloys have been found to be suitable for making into crucibles and dishes that will serve for many of the operations of the analytical laboratory, in place of the platinum that has been in use. Two of these will be mentioned.

"Palau" is a trade name for an alloy containing about 80 per cent gold and 20 per cent palladium. Its melting point is about 1370°.

"Rhotanium" is a name given to a series of gold-palladium alloys whose melting points range from 1150 to 1450°. Both palau and rhotanium may be used in place of platinum except where much oxidation is to be expected or where very high temperatures are employed.

Unfortunately the manufacturers discourage the use of these substitutes by maintaining the price of manufactured articles so close to that of platinum ware that the purchaser will usually pay the difference in order to obtain the more satisfactory platinum.

Burners.—The burner that is to be used by the analyst may be anything from the cheapest and simplest burner of the Bunsen type to the most expensive and complicated burner obtainable. The purchaser has his choice and probably certain advantages are possessed by each burner. The only feature that is really essential is independent regulation of air and gas supply. The requirements are quite different in different cases and the analyst must have at his disposal all kinds of flame, from the yellow illuminating flame to the most intensely hot and oxidizing flame, and he requires very small and very large flames of each class. In order to obtain this variety of flame there must be some method of regulating the gas supply without changing the pressure at the gas valve, since this also changes the amount of air drawn in at the mixer. The simplest form of Bunsen burner does not permit this gas regulation without unscrewing the upper tube and changing the gas jet by the use of pliers. Such regulation is not possible in practice.

In the Teclu burner (Fig. 13) the gas is controlled by the screw on the side of the base while the disc at the bottom of the cone controls the air supply.

In this burner the regulation of gas flow is not accomplished by altering the pressure under which it is delivered but by changing the size of the orifice in the burner. The maximum pressure is thus used at all times and the result is a better mixture of gas with air than is obtainable by regulating the gas cock of the supply line.

A very common error on the part of students lies in carelessness with regard to the regulation of flames. If a relatively cool flame is required and if a deposit of carbon is not objectionable the air should be excluded from the mixer. If, on the other hand, the highest efficiency of the burner is desired, careful regulation of the air and gas is necessary. The inner blue cone should be well defined and it should not show a yellow tip. If more air is admitted than that required to burn the gas completely with production of a blue flame, the result is a roaring and fluttering flame. This means that more air is being admitted than can be used and this air, in being heated by the flame, lowers the temperature of the latter.

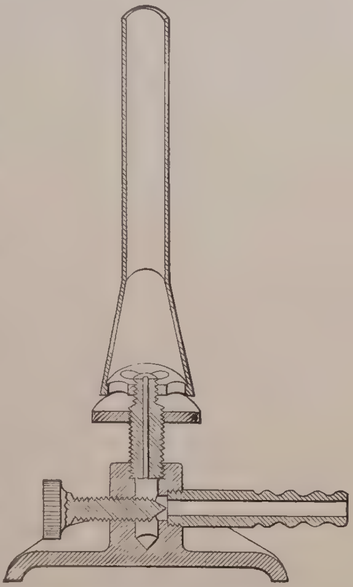

Fig. 13.—Section of Teclu burner.

Méker Burner.—A somewhat radical departure from the older types is found in the Méker burner. This is shown in section in Fig. 14. The air is drawn in through several holes in the base of the tube. The delivery of the gas under pressure into the inverted cone which forms the burner tube causes a greater reduction of pressure within the tube than is the case with burners having cylindrical tubes. The result is a greater inflow of air, making possible the combustion of a greater amount of gas in a given space, and also more complete mixing of gas and air.

The nickel grid through which the mixture flows at the top of the burner causes the gas to burn exactly as though each mesh were a small individual burner. The tip of the inner reducing cone of each small flame is usually about one millimeter above the top of the burner and, as all of the small flames unite to form one large one, the result is a highly concentrated flame, every part of which is oxidizing in character except a zone of about one millimeter in depth, immediately above the top of the burner. This is a distinct advantage, especially in heating platinum articles, since platinum is easily damaged by heating in a reducing flame.

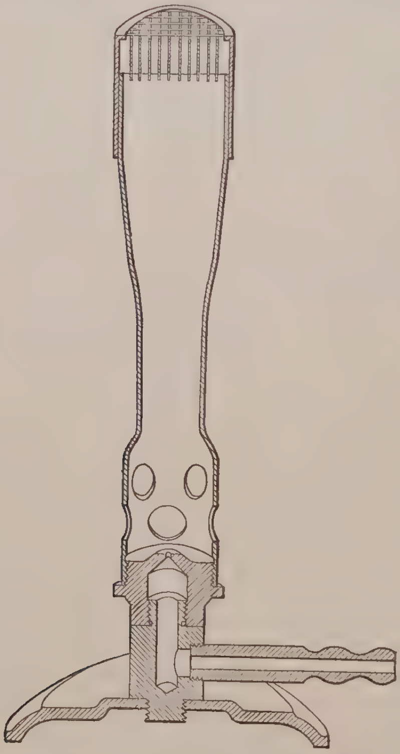

Fig. 14.—Section of Méker burner.

A number of imitations and modifications of the Méker burner are offered for use at this time. Most of these use the same combustion principle, the burners differing only in mechanical features.

Blast Lamp.—In order to produce a higher temperature a burner may be constructed so as to consume a larger quantity of gas, depending for its complete combustion upon admission of air under pressure. A burner so constructed is called a "blast lamp." Many forms of such burners are in use.

The flame of the Méker burner is nearly as hot as that of the ordinary blast lamp using the same gas and it may be substituted for the blast lamp in many cases. There is also a Méker blast lamp, similar in construction to the one already described but using air under pressure.

Weighing.—From all of the foregoing discussion it will be seen that every analytical determination involves, at some point, obtaining an accurate estimation of weights. Even the volumetric process requires weighing the sample and a weight is usually involved, directly or indirectly, in the standardization of the solutions used for the titrations. It is obvious from this that an accurately constructed weighing apparatus is a necessary part of the equipment of the analytical laboratory.

Methods of Weighing.—Any method that depends upon the attainment of equilibrium between the force of gravity and the resistance to distortion of a spring is necessarily subject to considerable and variable errors. These are chiefly due to variations in (*a*) elasticity of the spring and (*b*) the value of gravity for different altitudes. The only method that is free from these errors is weighing on a balance, a standard mass being compared with the object to be weighed and the former being varied until equilibrium is attained.

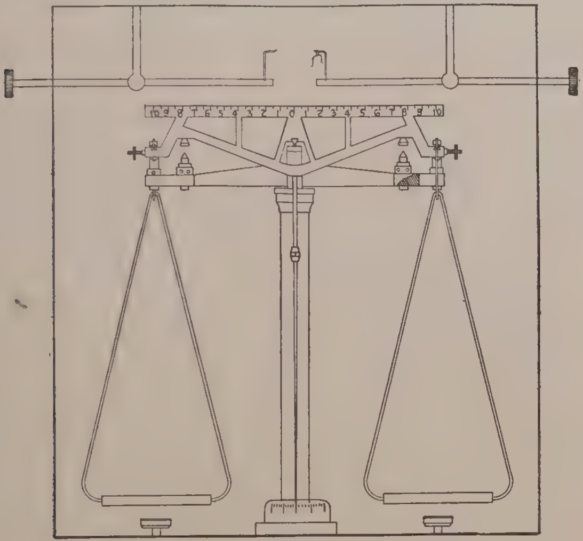

Fig. 15.—Essential parts of the balance.

The Balance.—The analytical balance should be so constructed as to provide means for accurate weighing to one ten-thousandth of a gram. In order that such weighing may be performed the balance must be constructed with mathematical accuracy. The three bearings are commonly of agate, ground to a very fine "knife" edge and each resting upon a smooth block of the same material. They must be so placed as to lie in the same plane while weighing (the central bearing is usually slightly below the plane of the end bearings, to allow for distortion of the beam when loaded) and absolutely parallel. The moving parts are as light as is consistent with the strength required to bear the rated load and they are provided with a mechanism for arresting their motion and for lifting the knife edges from their bearings. The entire balance is enclosed in a glass case, which is kept closed during the final adjustment of weights, so as to avoid interference of air currents.

These points will be made clearer by reference to Fig. 15, which shows only the skeleton of the balance.

Weights.—Practically all weighing operations of analytical chemistry are carried out by means of metric weights. A balance is rated for a certain maximum load and the largest piece of the set of weights should not be heavier than half this rated load. A balance rated to carry 100 gm in each pan will thus require a set of weights having a 50-gm piece as the largest piece of the set. The smaller pieces will then be, in grams, as follows: one 20, two 10's, one 5, one 2 and three 1's. These will total 100 gm. The fractional pieces (milligram pieces) are then apportioned as follows: one 500, one 200, two 100's, one 50, one 20 and two 10's, with a movable "rider" on the right arm of the balance beam to make another 10 mg, the beam being graduated so that by shifting the rider, 0.1 mg fractions may be made. It will be seen that these milligram pieces total 1 gm.

The Rider.—The reason for using a rider on the beam instead of the very small weight pieces on the pan is largely one of convenience. The rider may be adjusted with the balance case closed and this facilitates the final adjustment. This method also dispenses with the use of a large number of very small weights.

The actual weight of a rider to be used on a given balance will depend upon the manner in which the beam is graduated. These graduations are to indicate a certain number of milligrams and fractions. The generally approved method is to have the space between the central knife edge and the pan support marked in ten principal divisions, each with ten subdivisions. The number over the central pivot will then be 0 and that directly over the pan will be 10. If the rider is placed over the pan it will have the same value as if it were in the pan. *Hence it should weigh, in milligrams, whatever is indicated by this number.* Various balances have, instead of 10, the figures 5, 6 or 12 over the right pan. They will then require riders having these indicated values, in milligrams.

The Chain Rider.—The "Chainomatic" balance entirely dispenses with a separate rider. One end of a small gold chain is permanently attached to the balance beam. The other end of this chain is fastened to a hook which may be moved up and down a scale (Fig. 16), this action being controlled by a knob outside the balance case. Movement of the hook on the scale varies in a definite manner the length of side of the loop which is supported by the beam and this may be adjusted while the beam is in motion. This is a distinct advance in balance design, although this improvement adds considerably to the cost of the balance.

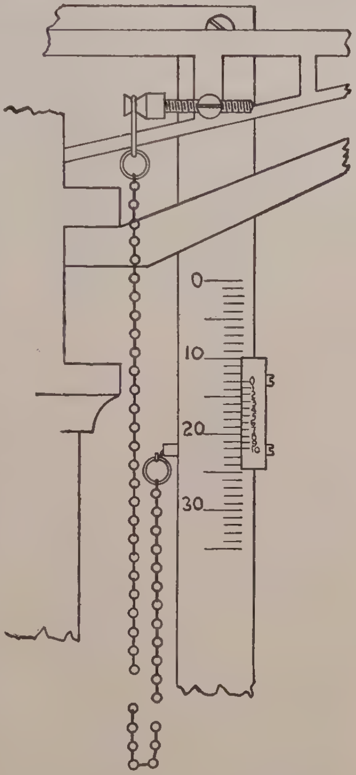

Fig. 16.—Chain rider and part of scale as used on the "chainomatic" balance.

Use of the Balance.—It has already been stated that the process of weighing involves the adjustment of weights upon one pan until they are in equilibrium with the material on the other pan. This is not done by noting when the balance beam fails to swing but by the more accurate method of causing it to swing several times in both directions, noting when a pointer attached to the beam swings equal distances on either side of a "zero point" on a fixed scale. The balance should be adjusted so that without load it swings about the true zero of the scale but thermal changes, settling of buildings, etc., will cause this to change and the zero point must be determined occasionally and the adjustment changed, if found necessary.

Differential Weighing.—Where the desired weights are found by a differential process it is not necessary that the adjustment of zero point should be made, or even that the zero point should be known. It is sufficient to assume the zero point to be the same as that of the scale. Although this may involve an error in weighing, this error will be the same for both weights obtained and the subtraction will eliminate it entirely. For example, a crucible is being weighed empty and again containing a precipitate of barium sulphate, in order to find the actual weight of the latter. A plus or minus error may have been made in the recorded weight, due to an incorrect assumption of zero point, but this will be the same for both weighings and when the observed weight of the empty crucible is subtracted from the observed weight of the crucible and barium sulphate, this error disappears.

To Determine the Zero Point.—Close the balance case and carefully lower the pan rests in such a manner as to stop any lateral swinging of the pans, then lower the beam rests and set the beam in motion by allowing the rider to rest momentarily on the beam, then raising it. This should cause the pointer to swing five to ten divisions on either side of the zero of the scale. Take at least three readings on one side and two on the other. Subtract the less average from the greater and divide the remainder by two. This gives the zero point if the proper direction is noted.

The zero point may be determined with sufficient accuracy for most work by simple observations without computations, by noting that the amplitude of vibration of the pointer diminishes regularly with each successive swing.

Weighing by the Single Deflection Method.—This rapid method has been described by Brinton.[1]

The pan rests must first be adjusted so that when released they shall give no swinging impulse to the system. That is, if the loads are in equilibrium there must be no swinging of the pointer at release of the pans, the beam rests being down. Equilibrium is then destroyed by adjusting one of the screws on the beam end, so that at release the pointer will swing 3 to 7 scale divisions in one direction. The point on the scale which the pointer reaches on its first excursion is taken as the "zero point," the pans having first been steadied to stop lateral swinging.

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[1] *J. Am. Chem. Soc.*, **41,** 1151 (1919).

In weighing, the weights are adjusted as by any other method, the rider finally being placed so that when the pans are released the pointer will reach the same "zero point," on its first excursion, that was first determined.

Although this method would seem, at first, to be essentially incorrect in principle, it is capable of giving accurate results in the hands of a careful analyst, with the following limitations:

1. It cannot be used with balances having a single control, releasing beam and pans at one operation.

2. The pan rests are cleaned, if necessary, with alcohol to prevent sticking to the pans, as otherwise a swinging impulse would be given by release of the latter.

3. Most balances show a variation of sensibility with variation of load. The "zero point" must then be determined at the approximate load that is to be weighed, if a single weighing is to be made, or at both loads in case of differential weighing, unless the single load or the difference between the two loads is quite small. One of these two conditions is met in most analytical work. Sample weights or weights of precipitates are less than one gram, in the majority of cases. If a sample is to be weighed on counterpoised glasses it is sufficient to determine the point reached on the first swing, with the empty glasses. If it is to be weighed from a weighing bottle, or if the precipitate is to be weighed in a crucible, the point reached when the filled weighing bottle or the empty crucible, respectively, is being weighed, is taken as the zero point for that particular pair of weighings.

4. It is obvious that a single observation gives no check upon chance causes of variation, such as vibration or air currents within the balance case.

The method is useful, especially for rapid work, if proper care and consideration are exercised. In any event the balance must be carefully tested at the beginning, to give assurance that it can safely be used for this method of procedure.

Calibration of Weights.—Expensive sets of weights are usually adjusted with sufficient accuracy for most analytical work, but with weights of the grade ordinarily available a calibration should be made. Weights that are found to be in error may then be either adjusted to accurate values or used with corrections.

This is a matter that is given serious attention in far too few laboratories, college or industrial. Commercial weights frequently are in error to the extent of two or three per cent and even larger errors may be found, after the weights have been in use for a year or more. To ignore such errors as these, while insisting upon a high degree of accuracy in the other phases of the laboratory work, is nothing less than gross inconsistency.

Calibration may be accomplished most conveniently and with accuracy by comparison of each piece of the set with the corresponding pieces of a standard set, whose corrections are known. Also, if the arms of the balance are known to be of the same length to within a negligible error $\left(\frac{r}{l} = 1 \pm \text{not more than } 0.00001\right)$ the comparisons may be made by placing the pieces on opposite pans of the balance and noting whether the rider must be used to obtain equilibrium and, if so, its necessary position on the beam. This gives, directly, the value of the experimental piece in terms of the standard piece. Since this is a direct comparison, the zero point of the balance must be known.

If an entire set of standard pieces is not available a single standard piece, as a gram, may be used, when the calculations become more complicated and the calibration less accurate. Also if the balance arms are not sufficiently near the same length (found by weighing an object and then exchanging object and weights, and reweighing) or if nothing is known regarding this point, a different method of comparison must be employed. This is known as the "method of substitution." *In the exercise that follows it will be assumed that an entire standard set is at hand and that the method of substitution is to be used.* This method is safest in any case and it makes unnecessary a determination of the relative length of the arms or of the zero point of the unloaded balance.

Calibration.—Besides the set to be calibrated there must be provided a standard set, also a third set to be used as counterpoise. This latter set may be of any cheap weights as the actual values of the pieces do not enter into the calculations.

Begin with one of the 1-gm pieces. Place a 1-gm counterpoise on the left pan and the corresponding standard piece on the right pan. Carefully lower the beam rests, and then the pan rests, and start the balance swinging by lowering the rider momentarily to the beam. Note the zero point and adjust the rider so that the pointer swings about the true zero of the lower scale. Now, without moving the rider, raise the pan rests at the moment when the pointer is passing zero, then the beam rests. Remove the standard piece and substitute the piece of the set to be calibrated. Repeat the determination of zero point. If the latter has not been changed by the substitution of weights, the standard piece and the experimental piece have the same value, irrespective of the value of the counterpoise. If the zero point has changed, shift the rider to restore equilibrium. The amount of shift gives the numerical difference between the two pieces. If the shift of the rider is to the *right* the experimental piece is *lighter* than the standard piece and if to the *left* it is *heavier*. Apply the indicated correction.

Repeat the process just described, comparing each piece of the entire set with the corresponding piece of the standard set, finally tabulating the corrections. These should be recorded on a card, which may be placed in the balance case for future reference.

If an accurately standardized complete set of weights is not available, the set may be calibrated to a single standard piece, or simply relative values of the various pieces may be established, using the method of Richards.[1]

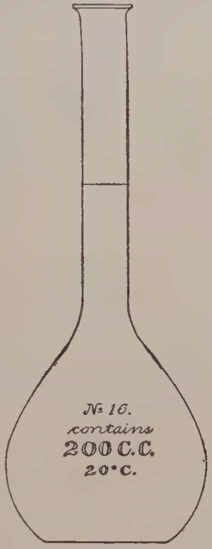

FIG. 17.—Approved shape and inscription for volumetric flasks.

Volumetric Apparatus.—It has already been shown that the balance is concerned, directly or indirectly, in all determinations made by volumetric processes. But the work is essentially different from gravimetric analysis in that no final weighing of precipitates is made. Instead of this a measurement is made of the volume of a standard solution required to complete a definite reaction with the substance under investigation.

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[1] *J. Am. Chem. Soc.*, **22,** 144 (1900); MAHIN, "Quantitative Analysis," 2nd ed., 66–69.

The apparatus necessary for this class of work will include accurately standardized volumetric flasks, burettes and pipettes. The first are for making solutions of definite concentrations and occasionally for measuring aliquot parts of such solutions. Pipettes are for measuring definite portions of solutions and burettes are for measuring the necessary volume of standard solution as the titration is being made. Because of the fact that the required volume of standard solution varies in different titrations and that it is unknown until the experiment is finished, the burette must bear graduations for small subdivisions, from zero to full capacity.

Figures 17, 18 and 19 illustrate the three types of apparatus just described.

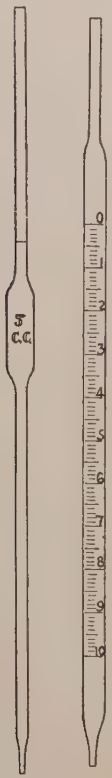

FIG. 18.—(*a*) Transfer and (*b*) measuring pipettes.

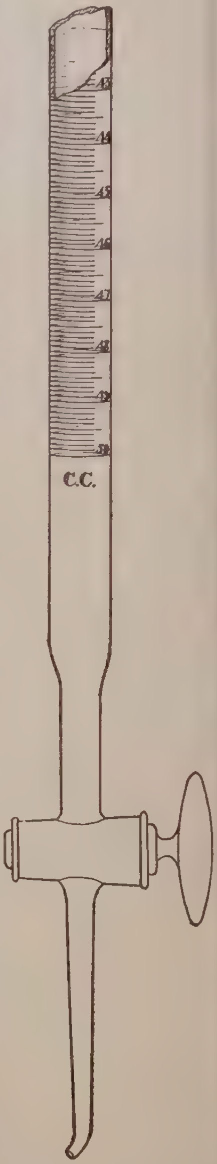

FIG. 19.—Showing tip and graduations approved for burettes.

Because of the fact that no glass apparatus can be made to deliver all of its contained solution upon emptying, it is necessary to specify whether a given piece of apparatus is graduated "to contain" or "to deliver" the stated amount. Also if the measurement of the delivered solution is to be at all accurate the apparatus must be constructed scientifically and used with many precautions. Much inaccurate work of the chemical laboratory may be traced to poorly constructed volumetric apparatus or to carelessness with regard to its use. The Bureau of Standards has undertaken a special study of this matter and has prescribed[1] rules for the construction and use of all volumetric apparatus. Some of the more important features of these specifications are given below. Every good laboratory should prescribe that apparatus shall conform to these specifications wherever possible and upon receipt of the various pieces they should be calibrated, in order to establish any necessary corrections in the graduations.

Specifications.—The **unit of volume** is the true liter. This is defined as *the volume occupied by one kilogram of pure water at* 4°. The **standard working temperature** is 20°.

Inscriptions.—Every instrument must bear a legend indicating the capacity in liters or milliliters (the latter is almost identical with cubic centimeters), the temperature at which it is to be used and whether to contain or to deliver the stated amount. Burettes and pipettes must bear a statement as to the time required for unrestricted outflow of the full quantity of water.

Special dimensions are given for each class of instrument.

The **time of outflow** is specified as follows: Pipettes having a single graduation ("transfer" pipettes) must have the tip of such size that the time of free outflow is not more than 1 minute nor less than the following, according to the size of the pipette:

Capacity (in cc) up to and including..............	5	10	50
Minimum outflow time (in sec.)......................	15	20	30

Burettes must empty in not more than 3 minutes nor less than as indicated below.

TABLE I.—RATE OF OUTFLOW FOR BURETTES

Length graduated, cm	Minimum time of outflow, sec.	Length graduated, cm	Minimum time of outflow, sec.
70	160	40	70
65	140	35	60
60	120	30	50
55	105	25	40
50	90	20	35
45	80	15	30

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[1] *U. S. Bureau of Standards, Circ.* **9**.

Calibration.—The standard working temperature is 20°. At this temperature 1 liter of distilled water, free from dissolved gases and weighed in air with brass weights, has an apparent weight of 997.18 gm. The simplest and most accurate method of calibrating is based upon this relation. Flasks or other apparatus, rated to contain any stated volume, are marked at the point reached by the meniscus of water, taken at the rate of 0.99718 gm for each cubic centimeter at 20°.

If the temperature of the balance room is not 20° a different weight of water must be taken. Bearing in mind that the apparatus actually has different volumetric capacities for different temperatures it will be seen that the calculated weight of water to be used for calibrating at temperatures other than 20° will include corrections for (*a*) expansion or contraction of glass, (*b*) change in density of water and (*c*) change in density of displaced air. All of these corrections are used in compiling the following table.

TABLE II.—TEMPERATURE CORRECTIONS

Temperature, deg.	Weight of water, in grams, to be taken for calibration
15	997.93
16	997.80
17	997.66
18	997.51
19	997.36
20	997.18
21	996.99
22	996.81
23	996.61
24	996.39
25	996.16

In calibrating burettes or pipettes the water is delivered from these into a weighing bottle, which is then stoppered and reweighed. The marking on a burette is too complicated to make it practicable to remark the instrument. Therefore the actual capacities between stated markings is calculated and a correction is applied, if necessary.

Cleaning Solution.—Prepare a cleaning solution by dissolving 5 gm of powdered commercial sodium dichromate in 500 cc of commercial sulphuric acid. The solution may be kept in a bottle having a wide mouth, such as those in which dry chemicals are purchased. Burettes may be inverted and left standing in the bottle, the solution then being drawn up by suction and held in the burette by closing the cock. For cleaning flasks the solution may be allowed to remain in the flask for some time or a small amount may be warmed and the flask rinsed with it. The chromic acid produced by the interaction of sulphuric acid and sodium dichromate oxidizes all organic matter and leaves the glass thoroughly free from it:

$$Na_2Cr_2O_7 + H_2SO_4 + H_2O \rightarrow Na_2SO_4 + 2H_2CrO_4,$$
$$4H_2CrO_4 + 3C + 6H_2SO_4 \rightarrow 2Cr_2(SO_4)_3 + 3CO_2 + 10H_2O.$$

Disappearance of the red chromate ion and the appearance of a green color, due to the positive chromium ion of chromic sulphate, indicate exhaustion of the solution.

Have the flask clean and quite dry. Place on a balance of capacity sufficiently great to carry the filled flask. Counterpoise, then add weights to the right pan at the rate of 997.18 gm for each liter. Remove the flask from the balance and fill with recently boiled distilled water at 20°, nearly to the point where it is thought that the mark will be placed. Remove drops from the inside of the neck, above the level of the water, using a roll of filter paper. Replace the flask upon the balance pan, then carefully drop in water from a pipette until the balance is in equilibrium.

To mark the flask cut a strip of gummed label, long enough to reach around the neck and about 5 mm wide. Carefully paste this with the original straight edge at the level of the meniscus, where the mark is to be made. Melt a small quantity of paraffin and brush a thin layer over the label and over a space of about 3 cm on either side of it. Using the point of a knife or of a sharpened piece of wood trace the straight edge of the label around the neck of the flask, making a mark sufficiently wide to be easily visible. The label here merely serves as a guide, making a regular line possible. Using a small feather as a brush apply a few drops of hydrofluoric acid and allow this to remain on the flask for two or three minutes, after which the acid may be washed off and the paraffin removed by warming.

In case the flask already has a graduation and the calibration shows this mark to be incorrectly placed it is desirable to indicate the new mark by making a small, well-defined arrow with the point resting exactly upon the new mark. The operator's initials may be placed beside the arrow and if this is done carefully, no interference will result.

If the flask contains no inscription etch the side in a manner similar to that shown in Fig. 17, page 41.

Calibration of Burettes.—The marking of a burette is too complex to be easily changed and the calibration will therefore consist of finding what, if any, corrections must be applied to the existing graduations.

First inspect the burette to determine whether it conforms to specifications, especially with respect to outflow time. If not, make what alterations are possible. A burette whose outflow time is too short will give erratic measurements. Clean the burette with cleaning solution, followed by distilled water. Fill with distilled water at 20°. Weigh accurately a 25 cc weighing bottle to the third decimal then measure 5 cc of water into it from the burette, and reweigh. Add another 5 cc and weigh, continuing until the bottle is full. Empty the bottle, reweigh and continue the process until the water from the entire graduated portion of the burette has been weighed. Repeat the process in order to have a check upon the work. Calculate the true capacity of each of the ten portions, using the weight 0.99718 gm for 1 cc of water. Record as follows, the capacities in the last two columns being recorded only as far as the second decimal place.

Mark	Weight of water, each interval	True capacity, each interval	True total capacity, zero to end of interval

Construct a curve showing the true reading at all points. In case any marked irregularity is observed at any part of the burette so that corrections taken from the curve would be inaccurate, recalibrate this portion, using 1 cc at a time.

Calibration of Transfer Pipettes.—Determine whether the time of outflow conforms to the requirements as set forth on page 43. If not, alter the tip of the pipette before calibrating. Provide a weighing bottle having a capacity of 10 cc, also a larger one having a capacity equal to that of the pipette. Cut a strip of paper about 2 mm wide and 5 cm long and carefully rule this in divisions of centimeters, marking from 0 to 5, and subdivisions of millimeters, using fine lines. Strips cut from coordinate paper are suitable for this purpose. Determine the approximate location of the capacity mark on the pipette by a rough experiment, unless the pipette is already marked. Paste the paper strip on the stem of the pipette with the division 2.5 at the supposed place for the capacity mark and with the zero toward the point of the pipette. Having cleaned the pipette with chromic acid solution it is drawn full of distilled water which is at a temperature of 20°, and the water is allowed to flow out until the zero mark is exactly reached. The pipette must be held in a vertical position and the eye must be in the same horizontal plane as is the meniscus. The pipette tip is now touched against the side of the beaker to remove the last drop. The finger is then removed from the top of the pipette and the water is allowed to flow, at full speed, into the larger weighing bottle, which has already been weighed. The tip is immediately touched to the side of the weighing bottle to remove the hanging drop. The weighing bottle is then stoppered and weighed. Calculate the volume of the water from the observed weight and record this as the capacity af the pipette to the zero mark.

Using the small weighing bottle determine in a similar manner the capacity of the pipette stem between 0 and 5. Divide this capacity by 50 in order to obtain the value of the smaller subdivisions.

From the capacities so determined calculate the number of stem divisions to be added to the zero in order to obtain the rated capacity of the pipette. Mark the point so determined, using the method directed for marking flasks.

CHAPTER III

QUANTITATIVE DETERMINATIONS

Comparative Usefulness of Different Methods.—In a general way it may be stated that gravimetric methods permit greater accuracy in determinations, while volumetric processes make for rapidity in routine work with large numbers of samples. This is due to the fact that one standardization of a solution forms the basis for many determinations, provided that a sufficient quantity of standard is made. Experience shows that, for a single determination, the time required to make and standardize a solution, added to that required to make a determination by means of this solution, often leaves the advantage in favor of the gravimetric method.

Whether the method used is gravimetric or volumetric, if precipitation is an essential part of the process it must be remembered that solubility is of the highest importance. No substance has zero solubility and, since the substance that is precipitating leaves a solution that is saturated with itself, the part in solution is necessarily not determined.

In the industrial laboratory methods are chosen, to a considerable extent, upon the basis of time saving. However it sometimes happens that for a given element or compound the known convenient and, at the same time, accurate methods fall in one class only. As an example of this may be mentioned the determination of the sulphate radical, which is almost universally carried out by precipitating and weighing barium sulphate.

Scope of the Laboratory Work.—The time that is available for the college laboratory course is, necessarily, inadequate to the gaining of the skill that comes from extensive experience. The entire field cannot be covered. The greater stress is therefore laid upon the learning of *principles* of correct manipulation as well as of chemical processes. The laboratory exercises that are described in Part I of this book are selected largely with this end in view. Most of the methods described are typical and illustrate the different kinds of work that will be of importance in the future activities of the agricultural chemist. In Part III these and many other methods will be applied to the analysis of materials that are of great importance to agriculture and, directly or indirectly, to the economic life of all of our people.

Certain conventional modes of expression are common and will be used in the following pages. Most of these are familiar to the student but the following should be especially noted:

(*a*) *Water* always means distilled water, unless otherwise stated.

(*b*) *Accurately weighed samples* are always understood, even when "about 2 gm," or a similar expression, is used, unless the use of an approximate weight is specifically directed.

(*c*) *Temperatures* are always centigrade unless otherwise specified.

CHLORIDES

Gravimetric, by Weighing Silver Chloride.—Silver chloride is a substance of very slight solubility in water and its precipitation and weighing therefore forms the basis for the determination of chlorine (of inorganic chlorides) and of silver. Silver chloride dissolves in pure water to the extent of about 0.0015 gm per liter. This corresponds to 0.0011 gm of silver or 0.0004 gm of chlorine. As the total amount of water that is used in the precipitation and washing processes need not exceed 150 cc, and can be made less than this, it may be seen that the recovery of chlorine or of silver may be regarded as practically complete, for all ordinary purposes.

However, it must be remembered that silver chloride dissolves easily in ammonium hydroxide and to an appreciable extent in concentrated solutions of sodium or potassium chloride, and of hydrochloric acid. It is decomposed by warming with sodium or potassium hydroxide, silver oxide being formed.

In the determination of chlorine in impure chlorides it is necessary to guard against the precipitation of other silver salts, such as phosphate or carbonate, by having a small excess of acid present. Nitric acid is suitable for this purpose and this serves also to insure against the objectionable action of bases, noted above. Silver nitrate being used as reagent, the following reaction occurs:

$$AgNO_3 + MCl \rightarrow AgCl + MNO_3.$$

The following experiments must be conducted in a room which is not brightly lighted and they should not be unduly prolonged.

Gravimetric Determination.—Prepare two "Gooch" (Caldwell) filters by the following procedure, first marking them I and II as directed on page 31: Place the crucible in the holder as shown in Fig. 5, page 25. Apply the suction and pour in prepared asbestos suspended in water until a felt of sufficient thickness is obtained on the perforated bottom. The required thickness will vary according to the condition of the asbestos, a comparatively fine material making a compact pad which need not be as thick as one of coarser material. These points must be determined by experiment, guided by the advice of the instructor. Place a small perforated porcelain plate on the pad, to prevent injury when solution is poured in.

Finally give the crucible a single rinsing with redistilled alcohol to promote rapid drying, drawing out as much liquid as possible. Remove the crucible, carefully wipe the outside and place in an oven which is maintained at a temperature between 105 and 110° and dry for at least 30 minutes. Place in the desiccator and weigh after 30 minutes cooling.

If an alundum crucible is to be used instead of the Gooch it is placed in the holder so that the top is even with the rubber. This is to provide for thorough washing of the entire body of the crucible, which is porous. No asbestos is used but a new crucible should be given a preliminary washing with hot water, followed by alcohol. It should then be dried at 105 to 110° before weighing.

While the filters are drying proceed with the weighing and precipitation processes. Fill a clean, dry weighing bottle with the powdered and well mixed chloride sample. Provide two clean, 250-cc beakers of Pyrex or other resistance glass and mark them I and II. If the substance is known to be unaffected by contact with air it may be poured directly into one of the counterpoised glasses on the balance, until about 0.2 gm is obtained. (The glasses should have been brought to balance by means of the rider.) This sample is then weighed accurately and brushed into one of the beakers by means of the small pencil brush of camel's hair. A second sample is weighed and brushed into the second beaker. The weights are recorded in the proper places in the data book.

If the nature of the sample is such that it should not be unnecessarily exposed to air it must be weighed by difference. Place the filled weighing bottle on the left pan of the balance, using for this purpose a pair of crucible tongs having short pieces of clean tubing drawn over the tips, and carefully weigh. Record this weight in the data book at the top of the space marked for sample I. Carefully remove the stopper, holding over beaker I, and pour about 0.2 to 0.5 gm into the beaker. Replace the stopper, using great care that no particles shall fall outside the beaker and be lost, then reweigh the bottle and contents. For these weighings the zero point of the balance need not be known, as explained on page 38. Record the last weight under the first and subtract to obtain the weight of sample used. Record the last weighing also at the top of the space for sample II. Remove a second portion to beaker II and reweigh the bottle, recording under the preceding weighing. Subtract again for the weight of sample II.

Dissolve each weighed sample in 75 cc of distilled water, measured with a fair degree of accuracy in a graduated cylinder, and add 1 cc of 20-per cent nitric acid. (Water and acid must be free from chlorides. Test by mixing the quantities mentioned above and adding a few drops of clear silver nitrate solution.) Heat the chloride solution nearly to boiling then precipitate with clear 5-per cent solution of silver nitrate, adding drop by drop from a pipette and stirring continuously. Ten to 20 cc of solution may be required, according to the nature and purity of the sample.

Cover the beakers and place on the steam bath or over a low flame which shows no yellow. Digest at near the boiling temperature until the precipitate has been well flocculated, then test for completeness of precipitation by adding a drop or two of silver nitrate solution to the clear, supernatant liquid.

During this process of digestion the crucibles are to be removed from the oven and cooled in desiccators for 30 minutes, then weighed accurately, handling only with clean crucible tongs (not rubber tipped). Place a crucible in the holder and, if a Gooch is used, moisten the pad with a few drops of water from the wash bottle. Apply the suction to either the Gooch or the alundum crucible and filter, holding the beaker close to the top of the crucible and pouring down a glass rod. If a Gooch crucible is used place the rod against the perforated plate covering the asbestos. Rinse all loose precipitate into the filter, then clean the beaker by means of the policeman (a rubber-tipped glass rod) and wash bottle and finally wash the entire precipitate and crucible by pouring in 2-per cent, chloride-free nitric acid from a beaker until the washings show no cloudiness with a drop or two of dilute hydrochloric acid, thus showing that all silver nitrate has been removed. In making such a test first rinse the *outside* of the lower end of the funnel tube, then collect about 1 cc of the washings from the silver chloride in a clean test tube containing a drop of dilute hydrochloric acid. If an alundum crucible is used, observe particular care in washing the upper portion, as the porous walls are filled with a solution containing all soluble salts present.

Finally give the silver chloride and filter a single rinsing with redistilled alcohol, wipe the outside of the crucible, if a Gooch, and dry at the temperature that was employed for the original filter. After 30 minutes, cool in the desiccator and weigh. Dry for additional periods of 15 minutes until the weight does not vary more than 0.3 mg. Calculate the per cent of chlorine in the sample.

Volumetric, by Titration with a Standard Solution of Silver Nitrate.—Chlorine of inorganic chlorides may be titrated very accurately by a standard solution of silver nitrate, potassium chromate serving as indicator. The solubility of silver chloride is so much less than that of silver chromate (1000 cc of water at 20° dissolves 0.0015 gm of silver chloride and 0.024 gm of silver chromate) that the latter exists permanently only after the chlorine has been practically completely precipitated. Its intensely purple color then serves as an indicator of the end point of the reaction with chlorides:

$$AgNO_3 + MCl \rightarrow AgCl + MNO_3; \qquad (1)$$

$$AgNO_3 + K_2CrO_4 \leftrightarrows Ag_2CrO_4 + KNO_3. \qquad (2)$$

This method may be employed to determine the chlorine of chloride *solutions* but the latter must be neutral before the titration can be made.

Equation (1), above, shows that the hydrogen equivalent of silver nitrate is 1 and its equivalent weight is therefore the same as its molecular weight. A tenth-normal solution (see page 7) will then contain 16.989 gm of the salt in each liter. Instead of this the solution may be made in the decimal system (page 8), each cubic centimeter being equivalent to some simple weight of chlorine. If n is used to indicate this required weight of chlorine, each liter of solution must contain $\frac{1000 \times 169.89\, n}{35.46}$ gm of silver nitrate.

Volumetric Determination: *Silver Nitrate Method.*—Prepare the following solutions:

(*a*) *Silver Nitrate.*—Calculate the weight of silver nitrate necessary to make 1200 cc of a solution, either tenth-normal or of such concentration that 1 cc is equivalent to 0.005 gm of chlorine, adding 1 per cent for possible impurities. Weigh to centigrams on counterpoised glasses and dissolve in chloride-free distilled water. Dilute to 1200 cc, mixing the diluted solution very thoroughly

(*b*) *Sodium Chloride.*—Prepare 500 cc of a solution of pure sodium chloride which has been powdered and dried at 105°. The concentration should be equivalent to that desired for the silver nitrate solution. The equivalent weight of sodium chloride being its molecular weight (58.46), a tenth-normal solution must contain 5.846 gm of the salt in each liter.

If, instead, 1 cc of the solution is to contain a simple, definite weight, n gm, of chlorine, each liter must contain $\frac{1000 \times 58.46\ n}{35.46}$ gm of sodium chloride. In this case n may conveniently be 0.005 gm. Whatever its value, n is the "chlorine factor", F_{Cl} (see page 5) of this solution.

Weigh the salt carefully on counterpoised glasses and dissolve in chloride-free water in a calibrated flask, dilute to the mark and mix thoroughly.

(c) *Potassium Chromate.*—Prepare 50 cc of a 5-per cent solution of potassium chromate and drop into it silver nitrate solution until a perceptible coloration, due to silver chromate, is obtained, thus showing that all chlorides have been removed. Allow the precipitate to settle and then decant into a bottle that can be stoppered.

Standardization.—Pipette 25 cc of the sodium chloride solution into a 200-cc casserole, or into a beaker which is placed over a white surface. Dilute with 25 cc of chloride-free distilled water, add 1 cc of chromate solution and then add the silver nitrate solution from a burette, stirring constantly, until the first permanent purple or reddish tint is obtained.

As each drop of silver nitrate solution is added, a purple precipitate is produced. Near the beginning of the titration this disappears immediately after mixing but toward the finish it persists for a longer time. This behavior serves as a warning of the approach of the end point. At the first indication of a faint but permanent color, rinse down the walls of the beaker or casserole with distilled water, read the burette and then confirm the reading by adding another drop of silver nitrate solution, which should deepen the color. When this has occurred, record the volume first noted as the end point. Rinse the casserole and repeat the titration with a new portion of sodium chloride solution, until agreement is obtained.

If a tenth-normal solution of silver nitrate is desired, the sodium chloride solution will have been made to this normality. In this case, use is made of the following simple relation: That the normalities of two solutions are inversely as the volumes found to be equivalent to each other. Suppose that 25 cc of tenth-normal chloride solution is exactly titrated by 24.15 cc of silver nitrate solution. The normality of the silver solution is then $\frac{25}{24.15} \cdot \frac{N}{10}$ and the "dilution ratio" is $\frac{25}{24.15} = 1.035$ (0.1035 is the "normality factor").

If the decimal system is to be used and if the sodium chloride solution has been made so as to contain 0.125 gm in 25 cc, as above suggested, the dilution ratio for the silver nitrate solution is again the inverse ratio of equivalent volumes, exactly as illustrated in the preceding paragraph.

In the above example 1 cc of the solution would have to be diluted to 1.035 cc, to make it tenth-normal, 100 to 103.5 cc, 1 liter to 1035 cc, etc. Upon the assumption that the sum of original solution and added water equals the volume of diluted solution (an assumption not strictly accurate, although practically so for solutions not more concentrated than these) 35 cc of water should be added to each liter of the standardized solution of this example.

The dilution is carried out as follows: Fill a dry 1000-cc volumetric flask exactly to the mark, add the necessary water from a burette and mix well. This requires a flask that will hold the required added water *above* the mark.

In case the dilution ratio has been found to be greater than about 1.010, as in this example, the dilution should be accomplished in two steps. The solution is first diluted, adding 3 or 4 cc of water less than the calculated amount. The solution is then mixed, restandardized and the final adjustment is made with greater accuracy.

Titration.—Weigh samples of about 0.2 to 0.5 gm of the chloride sample into 200-cc casseroles or beakers. Refer to the directions given on page 50 for weighing and recording weights. Dissolve the weighed samples in 50 cc of chloride-free water, add 1 cc of potassium chromate solution and titrate as in the standardization of silver nitrate solution. Multiply the number of cubic centimeters of standard solution required by its value in terms of chlorine, divide by the sample weight and multiply by 100, to obtain the per cent of chlorine in the sample.

Use of a Correction Factor.—There is a too common practice among chemists, and especially among industrial analysts, of using standardized solutions with a correction factor instead of diluting them to the desired concentration. In the example illustrated above the solution would be used as a tenth-normal solution, the factor 1.035 being used in the calculations of titrations to correct for the over-concentration. Or if a decimal solution were desired and if, for example, the first standardization showed the chlorine factor to be 0.005012 gm, instead of 0.005 gm, the calculations corresponding to Eq. (4) of page 5 would be

$$\frac{100\,V F_{Cl}}{S} = \frac{100\,V \times 0.005 \times 1.0024}{S} = \text{per cent Cl.}$$

This common technical error is based upon fallacious reasoning. In actual practice the standard solution is generally made in quantity for a considerable number of determinations, economy of time resulting from using one standardization for all. In such a case the solution should be diluted to the desired concentration, so that simplicity of calculations may result from the use of the milligram-equivalent (in the normal system) or of the simple factor of one significant figure, such as 0.005 as in the case already considered, for the substance to be calculated. Even if the solution is to be used for only one or two determinations the use of the correction factor is illogical. In the equation above, 0.005 and 1.0024 are constants and they should be collected. *In other words they should never have been calculated for such isolated experiments, the original* 0.005012 *serving in their place.*

The same reasoning applies to the normal system. The conclusion is that for a solution to be used for only one or two determinations, neither the decimal nor the normal system should be adopted, unless the primary standard is the active material of the standard solution, so that it may be accurately weighed, as is the case with sodium chloride. In such a case no correction factor will be necessary.

Volumetric, by Titration against Sodium Carbonate.—Hydrogen chloride (hydrochloric acid) may be determined volumetrically by titration with standard base, such as potassium hydroxide, using methyl orange or methyl red as indicator, or by titration against a solution containing a weighed amount of sodium carbonate. These methods are included in the general group designated by the term "acidimetry." The following two methods are applicable to hydrochloric acid only, as the special representative of the more general group of chlorides.

Hydrochloric acid reacts with sodium carbonate in two stages:

$$HCl + Na_2CO_3 \rightarrow NaHCO_3 + NaCl; \qquad (1)$$

$$HCl + NaHCO_3 \rightarrow H_2CO_3 + NaCl. \qquad (2)$$

This is followed (unless the concentration is small) by decomposition of the carbonic acid so produced:

$$H_2CO_3 \rightarrow H_2O + CO_2. \qquad (3)$$

For sodium bicarbonate, produced by reaction (1), and in concentrations ranging up to about fifth-normal, P_H = about 8.3. This is a nearly neutral solution and phenolphthalein, whose color range is 8.3 to 10, will indicate this point by the disappearance of a pink tint but titration by use of this indicator is not satisfactory because of the difficulties attending the prevention of local action according to Eq. (2), and consequent escape of carbon dioxide. At the completion of the second reaction the solution may contain any quantity of carbon dioxide up to the saturation point at the prevailing temperature. A tenth-normal solution of this gas, has a P_H value of about 3.75, from the small amount of weakly ionized carbonic acid existing in the solution. More concentrated solutions, such as might be produced by temporary supersaturation, will have somewhat lower P_H values but they will usually fall within the color range of methyl orange, which is 2.9 to 4. It is therefore convenient to titrate a weighed sample of sodium carbonate, dissolved in water, to an end point with methyl orange, this representing complete decomposition of the carbonate. In this case the hydrogen equivalent of sodium carbonate is 2, since both univalent sodium atoms are replaced by hydrogen. Its equivalent weight is therefore $\frac{106}{2} = 53$, while that of hydrochloric acid is 36.468, as usual.

These points will be made clearer by reference to Fig. 1, page 14.

The determination above discussed is introduced here as an example of a method for determining the concentration of any hydrochloric acid solution. The analysis of such a solution is expressed as *grams per cubic centimeter* or as *per cent by weight.* However, it should be remembered that the method will apply to similar determinations of concentration of other strong acids, such as sulphuric and nitric acids. Also it is a much used method for *standardizing* volumetric acid solutions, in which case the result of the experiment is expressed either in normality or in terms of the weight of some other element or group of elements equivalent to 1 cc as explained on page 8 and as discussed in connection with standard silver nitrate solution, above. This method for standardizing hydrochloric acid is described on page 83, for the analysis of carbonates.

Volumetric Determination: *Sodium Carbonate Method.*—The laboratory stock of "dilute" acid is suitable for this exercise, or a sample may be furnished by the instructor. Calculate the dilution necessary to make the solution approximately fifth-normal, if anything is known regarding the approximate concentration of the sample. If there is no available information on this point determine the specific gravity with a floating hydrometer (see page 97) and calculate the approximate concentration from the following table.

TABLE III.—CONVERSION TABLE FOR SPECIFIC GRAVITIES

Specific gravity*	Per cent, HCl	Specific gravity*	Per cent, HCl
1.000	0.16	1.115	22.86
1.005	1.15	1.120	23.82
1.010	2.14	1.125	24.78
1.015	3.12	1.130	25.75
1.020	4.13	1.135	26.70
1.025	5.15	1.140	27.66
1.030	6.15	1.142	28.14
1.035	7.15	1.145	28.61
1.040	8.16	1.150	29.57
1.045	9.16	1.152	29.95
1.050	10.17	1.155	30.55
1.055	11.18	1.160	31.52
1.060	12.19	1.163	32.10
1.065	13.19	1.165	32.49
1.070	14.17	1.170	33.46
1.075	15.16	1.171	33.65
1.080	16.15	1.175	34.42
1.085	17.13	1.180	35.49
1.090	18.11	1.185	36.31
1.095	19.06	1.190	37.23
1.100	20.01	1.195	38.16
1.105	20.97	1.200	39.11
1.110	21.92		

* At 15°.

In carrying out the dilution the required amount of acid is measured in a dry volumetric flask. This is then poured into a 1000-cc volumetric flask, the smaller flask being rinsed several times with distilled water and the rinsings added to the solution in the larger flask. Dilute to the base of the neck of the 1000-cc flask and mix; finally dilute to the mark and mix thoroughly.

Sodium Carbonate.—The sodium carbonate to be used as a standard is best made from sodium bicarbonate, as this salt can usually be obtained in a high state of purity, so far as other interfering solids are concerned, the only impurities being water and normal sodium carbonate. By heating to about 300° the following reaction is produced:

$$2NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2.$$

At the same time water of crystallization is expelled and pure dry sodium carbonate remains.

Heat about 25 gm of high-grade sodium bicarbonate in an electric oven to 300° for three to five hours. A platinum dish is best for this purpose. Cool the product in a desiccator and preserve in a tightly stoppered bottle. On a counterpoised glass weigh exactly 5.300 gm of the pure sodium carbonate. Brush this into a dry funnel which rests in the neck of a 500-cc volumetric flask. Jar most of the salt into the flask and rinse down the remainder with distilled water. Remove the funnel, gently agitate until the carbonate is dissolved, then dilute to the mark and mix well.

Titration.—Fill two burettes with the respective solutions. Before proceeding with the titrations, practice reading the color changes as follows: Place 100 cc of distilled water in a beaker and add a drop of methyl orange and 0.5 cc of carbonate solution. Drop in acid until the last drop changes the tint from yellow to pink. Now, drop in carbonate solution until the yellow color reappears. Repeat the process until the color change can be observed when but one drop of either solution is added. It will aid in the next process if this solution is preserved and another prepared. the two showing the two colors of methyl orange. These are set aside for comparison.

Measure out exactly 25 cc of the sodium carbonate solution into a beaker or Erlenmeyer flask, placed on a white surface. Add a drop of methyl orange and then carefully run in acid solution from the other burette until one drop changes the color from yellow to pink. Record the volume of acid required to do this. In case the end point has been overstepped, add 5 cc more of the carbonate solution to that already in the flask and continue the titration. Finally record the volumes of both carbonate and acid.

Since the carbonate solution was exactly fifth-normal, 1 cc is equivalent to 0.007294 gm of hydrochloric acid. The concentration (in grams of HCl per cubic centimeter) of the titrated solution is then $\frac{0.007294\,V_c}{V_a}$, where V_c and V_a are the volumes of carbonate and acid, respectively, equivalent to each other, and that of the original sample is $\frac{1000 \times 0.007294\,V_c}{SV_a}$, where S is the volume of sample used for the dilution. *Grams per cubic centimeter* may be converted into *per cent by weight* by dividing by the specific gravity of the sample, measured at 20°. The *normality* of the titrated acid solution is obtained, if desired, as in the case of silver nitrate solution: $\frac{25}{V'_a} \cdot \frac{N}{5} =$ normality, V'_a being the volume of acid equivalent to 25 cc of the fifth-normal carbonate. For the original sample, normality $= \frac{1000 \times 25\,N}{5SV'_a} = \frac{5000\,N}{SV'_a}$.

Volumetric, by Titration with Potassium Hydroxide.—In case the number of determinations of hydrochloric acid to be made is small, no advantage is gained by the use of standard potassium hydroxide. This is because the most satisfactory method for standardization of this solution is by titration against an acid solution which, in turn, is standardized by the method used in the preceding exercise. As that standardization was, in effect, an analysis of the acid solution no further experimental work should be necessary. But because of the fact that potassium hydroxide and sodium hydroxide solutions have a wider applicability than do carbonate solutions, serving for the titration of a great variety of strong and weak acids as well as of acid salts, the base solutions are more often kept as standards and, as such, may be conveniently used for the determination of hydrochloric acid in solutions.

Volumetric Determination: *Potassium Hydroxide Method.*—Calculate the weight of solid potassium hydroxide necessary for 1200 cc of fifth-normal solution. Add 1 per cent for water and other impurities and dissolve the calculated quantity in recently boiled and cooled distilled water. The solid base need not be weighed on the analytical balance. Dilute the solution to about 1200 cc, mix and allow to cool to 20°.

Prepare an approximately fifth-normal solution of hydrochloric acid and titrate against fifth-normal sodium carbonate, as directed in the preceding exercise. As the acid is merely an intermediate in the process its normality need not be calculated. Measure out 25 cc of the base solution from a burette, add a drop of methyl orange or methyl red and titrate to a faint pink color with the acid solution. (Methyl red should not be used if the base contains more than very small quantities of potassium carbonate.) This is simply an indirect comparison of the normalities of base and carbonate solutions, and the value of the acid does not enter into the final calculations if equal volumes of base and carbonate were used, as is seen from the following:

Let V_a = volume of acid equivalent to 25 cc of carbonate solution and V'_a = volume equivalent to 25 cc of base. Then the normality of the base $= \frac{V'_a}{V_a} \cdot \frac{\mathrm{N}}{5}$.

Titration.—The standard base just prepared will serve for the titration of hydrochloric acid and of other strong and weak acids, also of many acid salts, such as acid sulphates. The titration is carried out as in standardizing the base, except that phenolphthalein is used as indicator for the weaker acids. In any case the *same indicator* must be used when titrating the base against the intermediate acid in standardizing, since the invariable presence of a small amount of carbonate in the basic solution gives a slightly different normality, as calculated from titrations in presence of different indicators.

The titrations may be carried out in either direction, base being added to acid or acid to base, provided that the same color tint is taken as the end point indication in all cases with a given indicator. However it is usually true that it is easier to judge the first appearance of pink than its final disappearance. This means that it is usually better to add acid to base in presence of methyl orange or methyl red, and base to acid in presence of phenolphthalein. Methyl orange is the only indicator that can be used satisfactorily for carbonate solutions.

SULPHATES

Gravimetric, by Weighing Barium Sulphate.—The basis for this method is the following reaction:

$$BaCl_2 + M_2SO_4 \rightarrow BaSO_4 + 2MCl.$$

Solubility.—The solubility of barium sulphate in water is quite low. At 20°, 1000 cc of water will dissolve about 0.0026 gm of the salt. This contains 0.00153 gm of barium and 0.00107 gm of the sulphate radical. The precipitation of barium sulphate is made the basis for the determination of either barium or sulphates. In either case it is necessary to maintain a slightly acid solution in order to avoid the possibility of precipitating other barium salts, such as carbonate, oxalate or phosphate, in case traces of these salts, or of their acids, are present in the sample or in the reagents. A slight excess of hydrochloric acid is used for this purpose.

Crystallization.—Because of the very small solubility of barium sulphate it precipitates almost instantaneously as the reagent (a soluble sulphate or barium chloride) is added. On this account it usually forms relatively small crystals and these may be so small as to pass through filters of ordinary density unless care is given to the precipitation process. The best conditions are provided by keeping the solution hot, adding the reagent drop-wise and stirring continuously. This is followed by a process of digestion, which serves to enlarge the crystals already formed, as explained on page 25.

Change of Weight of Barium Sulphate.—Considerable care must be exercised in burning the paper upon which barium sulphate has been filtered and in subsequent ignition of the precipitate to expel traces of moisture. If the temperature is allowed to rise to too high a point barium sulphate will gradually decompose, yielding sulphur trioxide and losing weight thereby:

$$BaSO_4 \rightarrow BaO + SO_3. \qquad (1)$$

On this account the blast lamp or Méker burner should never be used for heating the precipitate and the temperature should not be allowed to rise above that of dull redness.

On the other hand, errors may occur through partial reduction of barium sulphate by carbon monoxide or organic gases resulting from heating of the filter paper. Barium sulphide is thus produced and again the material loses weight:

$$BaSO_4 + 4CO \rightarrow BaS + 4CO_2. \qquad (2)$$

In order to avoid this reduction the temperature should be held at as low a point as will serve to accomplish the combustion of the paper and a plentiful supply of air must be maintained by inclining the crucible and cover, as directed on page 29. Even with these precautions some reduction may occur but if heating is continued for a few minutes after the carbon has disappeared, reoxidation will take place:

$$BaS + 2O_2 \rightarrow BaSO_4. \qquad (3)$$

If it should be suspected that either or both of the errors just discussed has occurred in any given analysis a correction may be made by adding a drop of dilute sulphuric acid to the precipitate after the first weighing, then gently reheating to expel the excess of acid and water, and reweighing. A gain in weight is taken as evidence that sulphide or oxide of barium was present in the first case. The second weight is then the correct one.

This addition of acid, with subsequent heating, also serves to correct any error that may have occurred in the determination of *barium*, through the occlusion of barium chloride by the precipitating barium sulphate but not in the determination of the *sulphate radical*. It will be seen that such occlusion would occasion a negative error in the determination of barium, but a positive one in the determination of the sulphate radical. Then in the first case sulphuric acid converts occluded barium chloride into barium sulphate and gives a precipitate of correct composition. In the second case barium chloride is an occluded *impurity* in the precipitate and its conversion to sulphate merely serves to increase the error. Therefore, when barium chloride is used as the precipitating reagent for sulphuric acid it is highly important that the precipitation should be carried out very slowly by adding the reagent drop-wise and stirring vigorously. This method serves not only to minimize occlusion of the reagent but also to prevent the formation of a very finely divided precipitate.

Determination of the Sulphate Radical.—Weigh duplicate samples of 0.25 gm of the sulphate into beakers and dissolve in 75 cc of distilled water. Add 1 cc of dilute hydrochloric acid, heat to boiling and add, drop-wise and with constant stirring, a clear 5-per cent solution of barium chloride until the sulphate is completely precipitated. Digest on the steam bath until the precipitate settles and the solution clears, then filter and wash with hot distilled water, testing the washings finally with dilute sulphuric acid to insure removal of barium chloride.

While the digestion of the precipitate is proceeding the crucibles should be prepared. New crucibles generally lose weight slightly during the first heating. Clean two porcelain crucibles and mark them with small symbols, I and II (small dots are best), using an ordinary pen and ink. Allow the ink to dry, then place the covered crucibles over a blast lamp or a No. 4 Méker burner and heat with the full flame for 30 minutes. Remove the flames and allow the crucibles to cool to below redness, then place them in the desiccators and, after 15 minutes standing, weigh accurately, handling only with the tongs. The rubber tipped tongs are conveniently used for the cold crucibles.

After the paper and precipitate has been washed free from soluble salts, drain thoroughly and then slip the paper up the side of the funnel and fold as shown in Fig. 9. Place the folded paper in the weighed crucible. The crucible is then inclined on the triangle, as indicated in Figs. 10 and 11, and the flame of the ordinary burner is applied, gently at first to avoid loss of precipitate by spattering. After the paper has become dry the temperature is raised, the burner being placed under the *bottom* of the crucible so that warm air, and not products of combustion, pass through the crucible. Proceed in this way until all carbon has been oxidized and the precipitate is white, but without allowing the crucible to become more than a dull red. When the precipitate is quite white the covered crucible is cooled in the desiccator for 15 minutes and weighed. The difference between this and the first weight represents barium sulphate, from which the per cent of the sulphate radical, of sulphur trioxide or of sulphur is calculated.

In order to confirm the accuracy of the work the covered crucible is heated for additional periods of 10 minutes and cooled and weighed after each heating. The weight should not change more than about 0.2 mg after such heating, unless the temperature has been carried too high. If any trouble has been experienced in obtaining constant weight it may be well to add a drop of dilute sulphuric acid to the cooled material, then to evaporate carefully over a flame, and finally to heat gently and reweigh. This will correct for the formation of barium sulphide or oxide, as already explained.

Volumetric, by Titration with Standard Base or Carbonate.—Just as the chloride of hydrogen (hydrochloric acid) may be determined by titration with a standard solution of a base or carbonate, so may the *sulphate* of hydrogen (sulphuric acid) be determined. It is obvious that both determinations, as well as all other acidimetric determinations, are measurements of ionizable hydrogen alone, and that they can be calculated only to this hydrogen itself or, if other acids are known to be absent, to the acid present—in this case sulphuric acid. Such titrations could not properly be regarded as determinations of the acid radical, since salts of the essential acid are almost invariably present in small and variable quantities.

Determination of Sulphuric Acid: *Potassium Hydroxide Method.*—Fifth-normal potassium hydroxide is prepared and standardized as in the determination of hydrochloric acid, page 59. The sample of sulphuric acid, being non-volatile, may be weighed in a flask or beaker, if an accurate balance of sufficient capacity is at hand, or it may be measured and the specific gravity determined, the weight then being calculated. The dilution and titration are carried out exactly as directed on pages 57 and 59. The calculation of grams per cubic centimeter, per cent by weight and normality differ from that for hydrochloric acid only in the equivalent weights used. Sulphuric acid, being a dibasic acid, has a hydrogen equivalent of 2 and its equivalent weight is one-half of its molecular weight.

CALCIUM

Gravimetric, by Weighing Calcium Oxide.—If a neutral or basic solution of a calcium salt is treated with a soluble oxalate, as ammonium oxalate, a reaction like the following occurs:

$$CaCl_2 + (NH_4)_2C_2O_4 \rightarrow CaC_2O_4 + 2NH_4Cl. \qquad (1)$$

After filtering and washing the calcium oxalate this is ignited:

$$CaC_2O_4 \rightarrow CaCO_3 + CO; \qquad (2)$$
$$CaCO_3 \rightarrow CaO + CO_2. \qquad (3)$$

The calcium oxide is then weighed.

The method is applicable only to soluble calcium salts and to calcium oxide, hydroxide or carbonate. The last three compounds dissolve in hydrochloric acid, with formation of water or carbonic acid as byproducts, and carbonic acid is expelled by heating. Calcium phosphate must be given a preliminary treatment to separate phosphoric acid, as otherwise the phosphate will reprecipitate as soon as the solution is made basic.

Solubility.—The solubility of calcium oxalate in water at ordinary temperatures is about 0.0050 gm per liter, expressed as the anhydrous salt, this containing 0.0016 gm of calcium. A slight excess of ammonium oxalate diminishes the solubility, as explained on page 24, so that the recovery is very good. It is necessary to precipitate from hot solutions in order to avoid the formation of very fine crystals.

Purity of Precipitate.—Examination of physical data will show that oxalates of all of the alkaline earth metals and of the heavy metals have comparatively small solubilities in water. If any of these metals are present it will therefore be necessary to effect a preliminary separation before calcium can be precipitated and recovered as pure oxalate. This will be given due attention later in the work but in the following exercises calcium is assumed to be the only metal present, with possible exceptions of the alkali metals.

Determination of Calcium: *Gravimetric Method.*—From a closed weighing bottle or on counterpoised glasses (according to the nature of the sample) weigh accurately two portions of about 0.2 to 0.4 gm of the prepared calcium compound, placing in 200-cc Pyrex beakers. Add 75 cc of water and 5 cc of a 10-per cent solution of ammonium chloride, the latter to prevent the precipitation of possible traces of magnesium.

If the calcium salt contains carbonate it will not be completely dissolved in water. In this case do not add ammonium chloride but provide cover glasses for the beakers and add 10 cc of dilute hydrochloric acid. Calcium carbonate will dissolve with effervescence. The covered solution is then boiled for a few minutes to expel carbon dioxide. Now remove the covers and rinse them and the upper portions of the beakers with a jet of distilled water, allowing all of the rinsings to run back into the beakers. Dilute to about 75 cc.

Having obtained a solution by either method add 15 cc of ammonium hydroxide (5-per cent ammonia). A distinct odor of ammonia should be perceptible after blowing away the vapors above the liquid. Heat nearly to boiling and add, from a pipette, a recently prepared saturated solution of ammonium oxalate, drop by drop and with constant stirring. Ten to 15 cc of solution may be required. Digest on the steam bath until the precipitate settles and test the solution above by adding another drop of oxalate solution.

When precipitation has been completed, filter on a paper of medium density and wash precipitate and paper with hot water until the washings test free from chlorides, as determined by allowing the washings to fall into a test tube containing silver nitrate acidified with nitric acid. Finally drain as well as possible, remove the paper from the funnel and fold as shown in

Fig. 9, page 29. Place in a porcelain or platinum crucible that has been ignited to constant weight, incline the crucible and burn the paper. When the precipitate is white place the crucible in an upright position, cover and heat with the full flame of the blast lamp or of the No. 4 Méker burner until, after cooling for 15 minutes in the desiccator, the weight is constant. If the former burner is used the first weighing may be made after 15 minutes of heating. If the Méker burner is used the precipitate should be heated for 30 minutes before the first weighing.

From the weight of calcium oxide found calculate the per cent of calcium in the sample.

Volumetric, by Titration with a Standard Solution of Potassium Permanganate.—Instead of igniting calcium oxalate and weighing the oxide the purified oxalate may be redissolved in sulphuric acid and the resulting oxalic acid titrated with standard potassium permanganate:

$$CaC_2O_4 + H_2SO_4 \rightarrow CaSO_4 + H_2C_2O_4; \qquad (1)$$

$$5H_2C_2O_4 + 2KMnO_4 + 3H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 8H_2O + 10CO_2. \qquad (2)$$

Although this constitutes a direct titration of oxalic acid it is indirect, so far as calcium is concerned, and the calculation of the latter can be accurate only (1) if the precipitation has recovered all of the calcium, (2) if the calcium oxalate has been well purified and (3) if the oxalic acid resulting from its decomposition by sulphuric acid has been recovered completely. The process of washing is then doubly important. If ammonium oxalate is left in the precipitate this will later yield oxalic acid and give a high result for calcium. On the other hand, if the paper is not well washed after sulphuric acid has been added, not all of the oxalic acid which has been yielded by calcium oxalate will be titrated, and the result will be low.

Standard Solution.—Permanganate solutions for this purpose may be made in the decimal system or in the normal system. If calcium is the only element to be determined the decimal system offers greater convenience, of course. If the solution is to be used also for the determination of iron and possibly of other elements, then the normal system offers advantages. In either case it is necessary to know the equivalent weights of calcium and permanganate and these cannot be calculated as simply as in cases already considered.

Apparent Valence.—In ordinary reactions of double decomposition the valences of the elements and radicals which are being transposed are measures of the respective reacting powers of these entities. This is not true for reactions of oxidation and reduction. Here the reacting power of a compound is determined by the extent of *change* of valence. As an illustration may be taken the reversible oxidation of hydriodic acid by ferric chloride:

$$2FeCl_3 + 2HI \rightarrow 2FeCl_2 + 2HCl + I_2.$$

The valence of iron in ferric chloride is 3 but this compound does not exchange three atoms of chlorine for an equivalent amount of another radical—it simply parts with one atom of chlorine which oxidizes hydriodic acid. The hydrogen equivalent of ferric chloride in this reaction is then equal to the *change* in valence of iron. This is $3 - 2 = 1$.

For the purpose of this inspection the actual valence of the elements undergoing the reaction need not be considered as this depends upon the structural composition of the compounds, which is not always known. The *apparent* valence is that which is indicated by the simplest *direct* combination of positive and negative elements and it is therefore a measure of combining power. In the above reaction iodine is the element that is oxidized and it is sufficient to regard its apparent valence in hydriodic acid as 1 and in the form of the element as 0. The hydrogen equivalent of hydriodic acid is the difference between these two apparent valences, or 1.

The reaction between potassium permanganate and oxalic acid may be inspected similarly. Omitting the coefficients given in Eq. (2), page 65, the empirical equation is

$$H_2C_2O_4 + KMnO_4 + H_2SO_4 \rightarrow K_2SO_4 + MnSO_4 + H_2O + CO_2.$$

Obviously, carbon is the element that is oxidized and manganese is reduced. The apparent valence of an element which is in the negative radical of an oxyacid or of its salt is found by subtracting the total valence of the positive radical from that of the other elements of the negative radical and dividing the result by the number of atoms of the element in question.

In oxalic acid the apparent valence of carbon is then $\frac{(4 \times 2) - 2}{2} = 3$, while in carbon dioxide it is 4. The hydrogen equivalent of carbon is then 1 and that of oxalic acid (containing two atoms of carbon) is 2. Since one atom of calcium is equivalent to one molecule of oxalic acid, the hydrogen equivalent of the former also is 2 and the equivalent weight of calcium is $\frac{40.07}{2} = 20.035$.

The apparent valence of manganese in potassium permanganate is $(4 \times 2) - 1 = 7$. In manganese sulphate it is 2. The hydrogen equivalent of potassium permanganate is then 5 and the equivalent weight is $\frac{158.03}{5} = 31.606$.

Standard Potassium Permanganate Solution.—In order to prepare a stable solution it is necessary that manganese dioxide shall be absent and that the container and the distilled water shall be free from reducing matter. Bottles and flasks are cleaned by a preliminary treatment with warm chromic acid cleaning solution, followed by a thorough rinsing. A good distilling process will usually produce sufficiently pure water; manganese dioxide already present in the permanganate is removed by filtering the solution through asbestos.

From the above discussion of equivalent weights it will be seen that a normal solution of potassium permanganate will contain 31.606 gm in each liter. A solution, 1 cc of which is equivalent to a given weight of calcium, will contain in each liter: $\frac{1000 \times 31.606\,n}{20.035}$ gm, where n is the weight of calcium to be equivalent to each cubic centimeter.

The solution may be standardized against pure sodium oxalate, ferrous ammonium sulphate, or calcium carbonate whose calcium content has been determined gravimetrically. The equations for the reactions of the first two with permanganate follow.

$$5Na_2C_2O_4 + 2KMnO_4 + 8H_2SO_4 \rightarrow 5Na_2SO_4 + K_2SO_4 + 2MnSO_4 + 8H_2O + 10CO_2; \quad (1)$$

$$10FeSO_4.(NH_4)_2SO_4 + 2KMnO_4 + 8H_2SO_4 \rightarrow 5Fe_2(SO_4)_3 + K_2SO_4 + 10(NH_4)_2SO_4 + 2MnSO_4 + 8H_2O. \quad (2)$$

(Ammonium sulphate of the double salt is seen to play no part in the reaction and its formula could be omitted from the equation. However, it must be considered in calculating the equivalent weight of the salt, since it is weighed along with the ferrous sulphate. The same is true of water of crystallization.)

For standardizing by calcium carbonate the latter is weighed and treated according to the principles discussed on page 65, the value of the permanganate solution being calculated from the volume of solution found to be equivalent to this weight.

The equivalent weights of all of these substances may be calculated by the methods illustrated in the preceding discussion.

Preparation of Solution.—Prepare 1200 cc of a solution, either tenth-normal or of such concentration that 1 cc is equivalent to 0.002 gm of calcium, as the instructor may direct, as follows: Weigh to centigrams on counterpoised glasses, 1 per cent more than the calculated amount of the best grade of potassium permanganate obtainable, brush this into a glass stoppered bottle and add 1200 cc of water. Agitate until the salt is thoroughly dissolved and the solution is well mixed. Place the bottle out of bright light for 24 hours then decant through a Gooch filter or an alundum crucible into a cleaned flask or bottle, using a pump. The solution must not be allowed to come into contact with rubber. Rubber stoppers used during the filtration process should first be well washed to free them from loose material. Do not attempt to recover the last portions of solution remaining in the bottle.

Standardization.—Use one of the following methods.

(*a*) *With Sodium Oxalate.*—Weigh two or three portions of about 0.2 gm each of sodium oxalate of known purity (a Bureau of Standards sample if this is available) into 250-cc Erlenmeyer flasks. Dissolve in 50 cc of recently boiled and cooled distilled water and add 10 cc of dilute sulphuric acid. Place a thermometer in the flask, warm to 90° and titrate with permanganate solution, stirring vigorously and continuously. The permanganate must not be added more rapidly than 15 cc per minute and the last cubic centimeter must be added drop-wise, with particular care to allow the color from each drop to disappear before the next drop is added. When a final permanent pink is obtained observe the volume of solution required and calculate (*a*) the normality of the permanganate solution and (*b*) the weight of calcium equivalent to each cubic centimeter, referring to the discussion on page 67.

(*b*) *With Ferrous Ammonium Sulphate.*—Use accurately weighed portions of about 1 gm of the pure crystallized salt. Titrate as for sodium oxalate except that the solution is not to be heated and the titration may be carried out more rapidly, the reaction being nearly instantaneous. The experiment must be completed immediately after dissolving the iron salt, as otherwise oxidation by air will vitiate the results.

(*c*) *With Calcium Carbonate.*—Use a dried sample in which calcium has been determined gravimetrically as directed on page 64. The method to be used for standardizing the permanganate solution is exactly the same as described below for the determination of calcium, and from the results the normality and the calcium equivalent of the solution are calculated:

$$\frac{WP}{V} = \text{calcium equivalent of the permanganate, and}$$

$$\frac{WP}{0.020037V} = \text{normality of permanganate, where}$$

W = weight of calcium carbonate,
P = known proportion of calcium in the carbonate,
V = volume of permanganate solution used, and
20.037 = equivalent weight of calcium.

If *pure* calcium carbonate is used, P = the factor for calcium in calcium carbonate, divided by 100.

Determination of Calcium: *Permanganate Method.*—Weigh on counterpoised glasses two portions of the prepared sample into 250-cc Pyrex beakers, using 0.2004 gm if the standard solution is tenth-normal, or exactly 0.2000 gm if the solution is in the decimal system. (See the discussion of factor weights, page 8. It is not always advisable to use this system for such small samples. If not, weigh accurately samples of *about* 0.2 gm). Add 75 cc of water, cover and add 10 cc of 10-per cent hydrochloric acid. After the material has dissolved boil the covered solution gently to expel carbon dioxide, then rinse down the cover glass and the sides of the breaker with distilled water. Add 15 cc of ammonium hydroxide (5-per cent ammonia), which should leave the solution distinctly basic but clear.

Heat nearly to boiling and add from a pipette 10 to 15 cc of a recently prepared saturated solution of ammonium oxalate, drop by drop and with stirring. Cover and digest on the steam bath until the precipitate settles, then test the clear, supernatant liquid for completion of precipitation by adding another drop of oxalate solution. Finally filter and wash with hot water until a few drops of the washings give no turbidity with a basic 1-per cent solution of calcium chloride, showing the complete removal of ammonium oxalate. In applying the wash water the entire paper must be treated, as in other cases.

Place a 250-cc Erlenmeyer flask under the funnel, pierce the point of the paper and wash as much as possible of the precipitate into the flask with a stream of hot water. Dissolve the remaining precipitate with two or three 5-cc portions of hot 25-per cent sulphuric acid. Finally give the paper a thorough washing with hot water from the wash bottle. The total volume of solution in each flask should be 50 to 75 cc.

Place a thermometer in the flask and warm the solution to 90°, then titrate in the manner described for the standardization of permanganate by sodium oxalate, page 68. If the sample weight was exactly 100 times the calcium equivalent of the standard solution, the burette will read directly in per cent of calcium.

IRON

Iron is sometimes determined gravimetrically by precipitating ferric hydroxide by ammonium hydroxide, igniting and weighing this as ferric oxide:

$$FeCl_3 + 3NH_4OH \rightarrow Fe(OH)_3 + 3NH_4Cl; \qquad (1)$$

$$2Fe(OH)_3 \rightarrow Fe_2O_3 + 3H_2O. \qquad (2)$$

However ferric hydroxide is a colloidal substance which adsorbs soluble salts with great tenacity, so that it is difficult to purify any but a small precipitate by washing. Also during ignition on paper, reduction of the oxide is liable to occur. For these reasons volumetric methods are usually preferable. Standard solutions of permanganates or of dichromates are suitable for this purpose.

Volumetric, by Permanganate.—In acid solutions potassium permanganate oxidizes ferrous salts by the following reactions:

$$KMnO_4 + 5FeCl_2 + 8HCl \rightarrow KCl + MnCl_2 + 5FeCl_3 + 4H_2O; \qquad (1)$$

$$2KMnO_4 + 10FeSO_4 + 8H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 5Fe_2(SO_4)_3 + 8H_2O. \qquad (2)$$

The equations show that the added acid plays a definite part in the reactions and if an insufficient amount is present the solution will become basic and a precipitate will form, consisting of hydrated manganese dioxide and basic iron salts:

$$3FeCl_2 + KMnO_4 + (n + 2)H_2O \rightarrow 3FeCl_2OH + MnO_2{\cdot}nH_2O + KOH. \qquad (3)$$

Aside from the trouble experienced in reading the end point, caused by the appearance of the precipitate, it is seen from Eq. (3), above, that manganese is reduced to the apparent valence of 4 instead of 2, so that the iron per cent cannot be calculated when both reactions take place.

In presence of hydrochloric acid more permanganate than the theoretical amount may be used, with liberation of some free chlorine. This action may be almost entirely prevented by the addition of manganese sulphate to the solution. Generally phosphoric acid also is added to prevent the hydrolysis of ferric chloride, thus avoiding the appearance of a red color which would mask the end point of the titration.[1]

Reduction of the Iron Solution.—Iron exists in the ferric condition in most ores or other minerals. In order to reduce the solution of ferric salt either stannous chloride, zinc, sulphurous acid or hydrogen sulphide may be employed. The first two are the only ones now commonly used.

Stannous chloride, in solution, possesses the advantage of instantaneous action if added to the hot solution of ferric chloride. If the iron is to be reduced by stannous chloride an addition of this salt to the ore during the process of solution will materially hasten the action. For the final reduction the stannous chloride solution may be added from a pipette, the disappearance of the red color of basic ferric chloride providing an approximate indication of the end-point.

In the analysis of iron ores there is occasionally trouble at this point unless certain precautions have been taken. In the first place, many iron ores contain appreciable quantities of organic matter and this serves to produce a yellow color when the ore is dissolved. As color due to this cause does not disappear when the iron has been reduced it is not possible to determine when the correct amount of stannous chloride has been added. This trouble may be avoided by igniting the weighed sample for a short time in a porcelain crucible, before dissolving.

The second cause of irremovable color comes from fusion of insoluble residues in platinum crucibles. The pyrosulphate which is used as a flux dissolves traces of platinum and this, with stannous chloride, forms a yellow solution containing a complex of tin and platinum. This interference is avoided by the substitution of porcelain crucibles for those of platinum.

After a slight excess of stannous chloride has been added the solution is cooled and a considerable excess of mercuric chloride is added, the unused stannous chloride being thereby oxidized:

$$2HgCl_2 + SnCl_2 \rightarrow SnCl_4 + 2HgCl.$$

Mercuric chloride will not oxidize ferrous chloride and hence may be left in the solution. If an insufficient excess of mercuric chloride is used, or if it is added too slowly, free mercury may be produced:

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[1] For the explanation of these points see Mahin, "Quantitative Analysis," 2nd ed., pp. 244–246.

$$HgCl_2 + SnCl_2 \rightarrow SnCl_4 + Hg.$$

The indication of such action is the appearance of a gray precipitate of mercury instead of the characteristic white silky crystals of mercurous chloride. If mercury is so produced the determination is ruined because this mercury will itself reduce some of standard oxidizing solution during the process of titration of the iron.

Standard Solution.—The permanganate solution as used for calcium is suitable for the iron determination, or a new solution may be made in the decimal system. In the latter case a concentration such that 1 cc is equivalent to 0.005 gm of iron is conveniently used. As iron is oxidized from a valence of 2 to a valence of 3 its equivalent weight is 55.84 and 1 cc of a tenth-normal solution is equivalent to 0.005584 gm of iron. The equivalent weight of potassium permanganate is one-fifth of its molecular weight, as was shown on page 67. The concentration of permanganate solution to be equivalent to any specified weight of iron may be calculated by the methods illustrated on page 67.

Determination of Iron in an Ore.—Sample the ore and grind the last selection to pass the 100-mesh sieve. Weigh three samples of exactly 0.5 gm of ore on the counterpoised glasses, brushing into each of three porcelain crucibles. Heat the crucibles without covers for 5 minutes, using the ordinary desk burner, then allow the crucibles to cool, place in casseroles and add to each 25 cc of concentrated hydrochloric acid. Add also at this point 0.5 cc of 5-per cent stannous chloride solution. Cover and warm until solution is complete or until no further action appears to take place. If the residue is not colored, proceed, without filtration, as directed below. If the residue is colored it may contain iron. In this case filter on a small paper and wash the paper free from iron solution with hot water. Set the filtrate and washings aside and burn the paper at a low temperature in a porcelain crucible. If the residue is small in amount and apparently contains little silicious matter it may be decomposed by fusing with potassium pyrosulphate. Cool and dissolve the mass in hot water, adding the solution to the former filtrate.

Concentrate the iron solution, if necessary, to about 50 cc and transfer to a 1000-cc Erlenmeyer flask. While the solution is nearly boiling add, drop by drop from a pipette, a 5-per cent solution of stannous chloride until the ferric chloride has just been reduced, this being made evident by the disappearance of the red color. Add two drops more of stannous chloride solution then cool quickly by immersing the flask in running water. When cool add, *all at once*, 25 cc of a 5-per cent solution of mercuric chloride and mix well with the solution. The precipitate should be pure white mercurous chloride without a trace of gray mercury. Dilute to 500 cc with recently boiled and cooled distilled water and add 50 cc of a solution containing 144 gm of phosphorous pentoxide, 245 gm of sulphuric acid and 67 gm of crystallized manganous sulphate in each liter of solution. Titrate at once with standard potassium permanganate solution and calculate the per cent of iron in the ore.

By Dichromate.—In acid solutions ferrous salts are oxidized completely by dichromates. Potassium dichromate, a salt readily purified by crystallization, is generally used as a standard. The reaction between this salt and ferrous chloride is expressed by the equation:

$$6FeCl_2 + K_2Cr_2O_7 + 14HCl \rightarrow 6FeCl_3 + 2KCl + 2CrCl_3 + 7H_2O.$$

As in the oxidation of iron by permanganates the acid actually takes a part in the reaction and if an insufficient amount is present, a basic condition will result and a precipitate of basic salts of iron and of chromium will form.

Potassium dichromate possesses several advantages over potassium permanganate as a standard oxidizing agent. It is relatively more stable and therefore may be obtained in a state of uniform purity. This makes it possible to standardize solutions by direct weighing when the degree of purity of the salt has been established by analysis. The relative stability is the same with solutions and the standard solution can be kept almost indefinitely without changing its concentration. Potassium dichromate may also be used for the titration of iron and other reducing agents in presence of hydrochloric acid or chlorides, without oxidation of the latter taking place. This is a very decided advantage in the determination of iron since it makes possible the use of stannous chloride as a reducing agent without the addition of manganous sulphate and phosphoric acid. There is no indicator that can be added directly to the solution which is being titrated by potassium dichromate and the color of the standard solution is not sufficiently intense to be of any use for this purpose. The indicator that is commonly used is a very dilute solution of potassium ferricyanide, placed in drops on a white porcelain "spot plate." Drops of the solution are removed from time to time by means of a stirring rod and allowed to touch the drops of ferricyanide. So long as ferrous iron is present the blue of ferrous ferricyanide is apparent on the spot plate. When the last trace of iron has been oxidized there is produced on the plate only the light brown ferric ferricyanide. There being nothing in the appearance of the solution of the iron salt to indicate the *approach* to the end point, the titration is necessarily somewhat tedious unless a system is devised for rapid readings. Such a system is indicated in the next exercise.

Standard Solution.—The solution should be of such concentration that 1 cc is equivalent to 0.005 gm of iron. Calculate the weight of potassium dichromate necessary for 1000 cc of such a solution. If the salt is known to be pure, weigh exactly the calculated weight and omit further standardization. If it is not pure but its oxidizing power known from previous determinations, calculate the weight of impure sample required and use this weight. If nothing is known of the purity use 1 per cent more than the weight of pure salt required for 1200 cc of solution and standardize the solution as directed below. In any case dissolve the weighed salt and dilute to the proper volume. In case titration for standardization is to be omitted and direct weighing is to be made the basis for standardization, 1000 cc of the solution should be accurately made and poured into a dry bottle.

Standardization, if this should be necessary, is accomplished by titration against ferrous ammonium sulphate. Write and balance the equation for the oxidation of ferrous sulphate by potassium dichromate in presence of sulphuric acid, referring, if necessary, to the equation for the oxidation of the chloride, page 73. Calculate the weight of crystallized ferrous ammonium sulphate necessary to reduce 35 cc of the dichromate solution. Weigh four portions of exactly this weight into 250-cc beakers and dissolve each, *just before titrating,* in 50 cc of recently boiled and cooled water. Prepare a 0.01-per cent solution of potassium ferricyanide and place a drop in each of the depressions of a white porcelain spot plate. Add to the solution of ferrous ammonium sulphate 5 cc of dilute sulphuric acid, and titrate at once, as follows: To the first solution add the dichromate solution 5 cc at a time, stirring well after each addition, and test by removing a drop by means of the stirring rod and touching to a drop of potassium ferricyanide solution on the spot plate. The end point is reached when a blue color is no longer produced on the plate, after standing for 1 minute. (Dust or reducing gases will interfere by reducing traces of ferric chloride.) Titrate the second solution by adding 5 cc less than the amount of dichromate solution used in the first, then adding 1 cc at a time. Titrate the third solution by adding 1 cc less than the total used in the second, then adding 0.1 cc at a time. Titrate the fourth solution in the same manner and take the average of the last two titrations for permanent record. Calculate the value of the solution in terms of iron. Dilute to make 1 cc equivalent to 0.005 gm of iron.

Instead of weighing four portions of ferrous ammonium sulphate a standard solution may be made by dissolving ten times the required amount, adding 50 cc of dilute sulphuric acid and diluting to 500 cc with recently boiled and cooled water. Portions of 50 cc are then measured and titrated. The solution oxidizes upon exposure to air and it must be kept in a closely stoppered flask.

Determination of Iron in an Ore.—Prepare a sample of iron ore by grinding to pass a 100-mesh sieve. Weigh four portions of exactly 0.5 gm each, using the counterpoised glasses and brushing the ore into porcelain crucibles. Heat the inclined crucibles for 5 minutes over the desk burner, cool, place in casseroles and dissolve in hydrochloric acid, with or without the addition of stannous chloride. Reduce each solution just before titration, following the directions given for dissolving and reducing by method (*b*) of the permanganate method but do not add the solution of manganous sulphate and phosphoric and sulphuric acids. Dilute to 100 cc. The titration is carried out exactly as directed for standardizing potassium dichromate solution. Calculate the per cent of iron in the ore.

ALUMINIUM

The direct determination of aluminium is made by precipitating the hydroxide, changing this to oxide by ignition, and weighing the product:

$$AlCl_3 + 3NH_4OH \rightarrow Al(OH)_3 + 3NH_4Cl; \qquad (1)$$

$$2Al(OH)_3 \rightarrow Al_2O_3 + 3H_2O. \qquad (2)$$

If iron and aluminium occur together they are precipitated together and the product of ignition is a mixture of ferric oxide and aluminium oxide. In such a case the usual procedure is to weigh the oxide mixture, then dissolve and determine the iron volumetrically, calculating this to oxide and subtracting from the weight of mixed oxides to find the weight of aluminium oxide. Or the aluminium may be determined directly by precipitating as phosphate, first reducing the iron to the ferrous condition by sodium thiosulphate; ferrous phosphate is sufficiently soluble to make a separation possible. This method will be described later for the determination of aluminium in soils (page 258).

Solubility.—The solubility of aluminium hydroxide in water is not definite as this substance belongs to the colloid class. The presence of various salts diminishes the solubility to a low figure, and especially so if the solution is boiled to cause flocculation of the colloid. Either acids or bases will dissolve the precipitate; acids form soluble aluminium salts and bases form soluble aluminates:

$$Al(OH)_3 + 3HCl \rightarrow AlCl_3 + 3H_2O; \qquad (1)$$
$$Al(OH)_3 + NaOH \rightarrow NaAlO_2 + 2H_2O. \qquad (2)$$

The possibility of the second reaction makes necessary the use of ammonium hydroxide, rather than sodium or potassium hydroxide, for the precipitation, as the excess of ammonia may be removed by boiling the solution.

Determination of Aluminium.—Fill a weighing bottle with the powdered sample of an aluminium salt. Choose the method to be used in weighing according to the nature of the substance and weigh two samples of about 1 gm each into Pyrex beakers. Dissolve in 100 cc of water and add dilute, recently filtered ammonium hydroxide, stirring until the liquid is distinctly basic, as shown by a drop of methyl red added to the solution. Boil until the precipitate is coagulated and until the odor of ammonia above the solution is faint. Boiling after the odor has disappeared will cause some of the precipitate to return to the solution:

$$Al(OH)_3 + 3NH_4Cl \rightarrow AlCl_3 + 3NH_3 + 3H_2O.$$

Allow the precipitate to settle and then filter through paper, using a filter pump attached to a bell jar or filter flask and placing a supporting cone of hardened paper or platinum in the funnel. Wash with hot distilled water containing 1 per cent of ammonium nitrate, until the washings are free from chlorides, shown by adding a drop of nitric acid and a few drops of silver nitrate solution to a small amount of the washings caught in a test tube; also from sulphates, as shown by adding a drop of dilute hydrochloric acid and a few drops of barium chloride solution to another portion of the washings. Suck the precipitate as nearly dry as possible and transfer the paper and precipitate to a porcelain or platinum crucible which has been ignited and weighed, folding the paper in the manner already learned.

Heat very gently in the covered crucible until the moisture is volatilized, then raise the temperature and burn the paper, inclining the crucible and placing the cover as in the case of the ignition of the paper containing calcium oxalate. When all of the carbon has been burned, cover the crucible and heat over the blast lamp or the large Méker burner for 30 minutes. Cool in the desiccator and weigh. Heat again for 10 minutes, cool and weigh. If necessary repeat this process until the weight is constant.

Calculate the per cent of aluminium in the salt.

Aluminium oxide absorbs water from the air, reforming the hydroxide with a corresponding gain in weight. On this account the crucible and oxide should be weighed rapidly.

CARBONATES

The Carbonate Radical.—The determination of the carbonate radical of solid carbonates can be made with accuracy only by decomposing the carbonate with a stronger acid, then purifying the resulting carbon dioxide and absorbing it in some manner in another reagent. This is the basis for both gravimetric and volumetric methods. In the former class of methods the carbon dioxide is absorbed in a basic substance (usually potassium hydroxide or soda lime) contained in a small apparatus that can be weighed, the gain in weight serving as a measure of the quantity of gas absorbed. Or it is sometimes absorbed by a solution of barium hydroxide, the barium carbonate so formed being removed by filtration, dissolved in hydrochloric acid and the barium reprecipitated as sulphate. From the weight of barium sulphate the corresponding weight of carbon dioxide is calculated.

In the volumetric modifications the absorbing reagent is a standard solution of a base, such as barium hydroxide, a measured volume of this being titrated by a standard acid solution after the absorption is finished. Either method will give accurate results, if care is used.

Gravimetric Method.—The necessary parts of the apparatus are shown in Fig. 20. In this figure, *A* represents a generating flask in which the weighed sample of carbonate is placed. *B* is a dropping funnel having a capacity of 50 cc and having the lower end drawn out to a point and turned upward. This part should extend to the bottom of the flask. At the top of the dropping funnel a drying tube *C* is connected by means of a rubber stopper and a bent glass tube. The drying tube is filled with soda lime for the absorption of carbon dioxide from the air that is later to be drawn through. Following the generating flask is a short condenser *D* (the lower end of which should be beveled) and then U-tubes *E*, *F* and *G*. The first U-tube is omitted if sulphuric acid is to be used for decomposing the carbonate or it is filled with an absorbent for hydrochloric acid vapors if this acid is to be used. The U-tubes *F* and *G* are filled with granular calcium chloride which absorbs moisture from the gas mixture. Following these is the apparatus *H* in which potassium hydroxide is placed for the absorption of carbon dioxide. This apparatus also carries a small tube filled with calcium chloride to prevent the removal of moisture from the apparatus, which would occur if the dry entering gases were allowed to leave the apparatus saturated with moisture. To provide a means for drawing air through the whole apparatus the aspirator *J* is placed at the end of the series, while to prevent moisture from diffusing backward into the absorption apparatus the calcium chloride tube *I* is interposed.

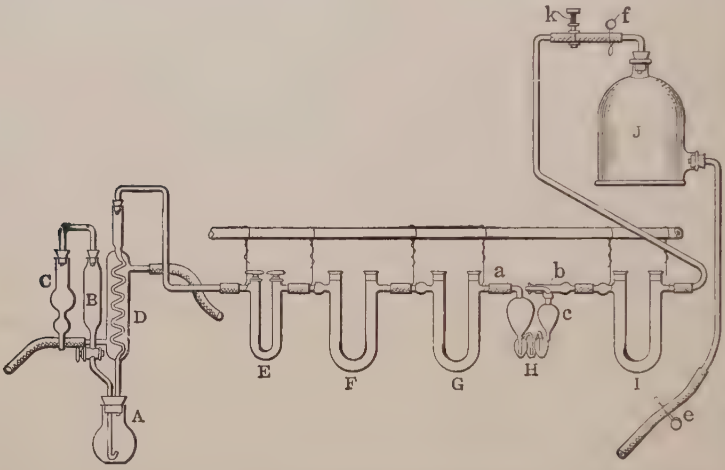

FIG. 20.—Apparatus for the gravimetric determination of carbon dioxide in carbonates.

Choice of Acid.—The carbonate should be decomposed with dilute sulphuric acid if insoluble sulphates are not thereby formed. This point may be decided by making a preliminary qualitative test. If this acid cannot be used, hydrochloric acid is taken for the purpose but it then becomes necessary to introduce into the apparatus train a tube containing silver sulphate for the absorption of traces of hydrochloric acid that might pass the condenser.

Absorbent for Carbon Dioxide.—A water solution of potassium hydroxide serves best for this purpose. This is made by dissolving one part, by weight, of the base in two parts of water, thus making a solution practically 33 per cent by weight. The containing apparatus, to be weighed before and after the absorption, must include a small additional tube which is filled with calcium chloride. This prevents loss of moisture from the weighed apparatus.

Soda lime is sometimes used for the absorption of carbon dioxide. This is made by fusing together sodium hydroxide and lime, the product being granulated during the cooling process. The chief objection to this use of soda lime is the fact that it is somewhat uncertain in its action and the absorption of gas is liable to be incomplete unless the moisture content is kept within fairly narrow limits (about 15 per cent).

Determination of the Carbonate Radical: *Gravimetric Method.*—Procure the following parts for assembling:

1 dropping funnel, 50 cc, with one-hole rubber stopper,
1 short, wide flask, 75 cc, such as is used for fat extractions, with two-hole rubber stopper,
1 condenser with body not more than 6 inches long,
3 U-tubes with corks to fit,
1 U-tube with glass stoppers,
1 straight drying tube with one-hole rubber stopper,
1 set Geissler "potash bulbs" or bulbs of some other approved form,
1 aspirator bottle, tubulated near bottom, with one-hole rubber stoppers to fit,
1 piece glass tubing, about 2 feet by ½ inch, for supporting apparatus,
2 clamps,
2 pinch cocks,
1 small screw clamp (Hoffman screw),
2 retort stands,
Glass and rubber tubing for connections.

Fill and connect the apparatus in the manner previously described. The drying tubes are filled nearly to the side arms with a good grade of granular calcium chloride. A loose plug of cotton is placed on top of the chloride and then a cork is pressed in until the top is about 2 mm below the top of the tube. Into the cup thus formed melted paraffin is poured as a sealing material. If bubbles appear in the paraffin after cooling they are removed by remelting the surface by a flame.

When filling the absorption bulbs with potassium hydroxide solution the latter should not be warmer than the air of the room. The bulbs are now detached from the apparatus and the solution is drawn in through a tube attached at *a*, suction being applied at *b*. The solution should about half fill the bulb *c* when air is bubbling through. The ground-glass joint between the drying tube *b* and the bulbs should be lightly coated with vaseline and the tube then twisted on until it fits closely enough that there will be no danger of loosening during the course of an experiment. Any surplus vaseline is removed from the outside of the joint.

Place the bulbs in position, close the cock of the dropping funnel and open the pinch cock at *e* to allow water to flow from the aspirator. Bubbles of air will at first pass through the bulbs but this action will finally cease unless there is a leak in the apparatus, in which case it must be found and closed. It is important that all glass tubes be brought entirely together inside the rubber connections since rubber is slightly permeable to gases.

After the apparatus has been shown to be free from leaks the pinch cock at *f* is closed, the cock of the separatory funnel is slowly opened and, after equilibrium is established, the clamp *k* is so adjusted that when clamp *f* is opened air will pass through the bulbs at a rate not greater than three bubbles per second. Clamp *k* is not thereafter changed. This provides against too rapid flow of gas under any conditions. Clamp *f* is now closed, the bulbs are removed, the inlet and outlet tubes are closed by short rubber tubes containing glass plugs and the bulbs are wiped clean and placed in the balance case. A short glass tube is inserted to bridge the gap made by removing the bulbs.

The bulbs should be allowed to stand for 15 minutes before weighing. In the meantime about 1 gm of the carbonate is weighed and brushed into the generating flask and a small amount of water is added to moisten the sample. The stopcock of the funnel *B* and the clamp *E* are now opened and 500 cc of air is drawn through the apparatus, measured by the outflowing water from the aspirator. This frees the apparatus from carbon dioxide. After the absorption bulbs have stood for 15 minutes the tubes carrying the plugs are removed and the bulbs are weighed. The plugs are then replaced and left so until the bulbs can be connected in the apparatus. Fifty cubic centimeters of dilute sulphuric acid or hydrochloric acid is placed in the dropping funnel, a test having previously been made to determine whether sulphuric acid will form a clear solution with the carbonate. If such a solution is not produced, of course hydrochloric acid must be used and silver sulphate and pumice must be placed in tube *E*.

Reconnect the apparatus and open all cocks except the stopcock in the dropping funnel, leaving the clamp *k* set for the proper rate of gas flow, as previously determined. Slowly open the cock of the dropping funnel, allowing acid to drop just fast enough to evolve carbon dioxide at the prescribed rate. The constant attention of the operator is necessary at this point, for by causing too rapid evolution of gas some moisture may escape absorption in the small tube of the absorption bulbs and the experiment be rendered worthless.

The acid should be allowed to run in until about 1 cc is left above the stopcock, this acting as a seal during the subsequent boiling. After the decomposition of the carbonate is complete the solution in the flask is slowly heated until it boils, always with due regard to the rate at which the gas is made to flow through the absorption bulbs. The boiling is continued for one minute, when the flame is withdrawn, the cock of the dropping funnel being opened at the same time to allow air to enter so that no back suction occurs, due to the cooling effect. Air is now drawn through the apparatus until 1000 cc of water has flowed from the aspirator. This amount of air should be sufficient to sweep all of the carbon dioxide into the absorption bulbs.

The clamp *f* is now closed, and the absorption bulbs are removed, plugged and placed in the balance case. After 15 minutes they are weighed without the rubber tubes and plugs, the increase in weight being the weight of carbon dioxide. From this and the weight of sample the per cent of carbonic anhydride (combined carbon dioxide) or of the carbonate radical is calculated.

For the duplicate or any subsequent determination the generating flask and the dropping funnel are washed absolutely free from acid, so that no decomposition of the next carbonate sample may occur before the bulbs are in place.

If a large number of determinations are to be made with the same apparatus much time will be saved by providing two decomposition flasks and two absorption bulbs. While one determination is being made another sample may be weighed into the duplicate flask and the second absorption bulb may be weighed. The next determination may then be started while the first bulbs are standing in the balance case, preliminary to the final weighing. It is also necessary to determine when the various absorbents have become so saturated as to be inefficient for further work. Soda lime in the tube *C* is good until the lumps have begun to fall into a powder. Silver sulphate in the pumice of tube *E* may become inefficient through absorption of hydrochloric acid or through the accumulation of water in the tube. The solubility of silver sulphate in water is much less than in concentrated sulphuric acid. If the acid solution becomes diluted the silver salt crystallizes and will not thereafter readily absorb hydrochloric acid. As the silver sulphate becomes saturated with hydrochloric acid it darkens, on account of the action of light. When the darkening effect has proceeded as far as the middle of the tube the material should be replaced. Calcium chloride must be replaced when it becomes visibly moist *for the first third* of any absorbing tube.

Gravimetric by Absorption and Subsequent Precipitation.—Carbon dioxide, evolved by the method just described, is sometimes absorbed in a somewhat concentrated solution of barium hydroxide, the barium carbonate thus formed being then removed by filtration, washed, redissolved in hydrochloric acid and the barium precipitated as sulphate. The weight of this gives a measure of carbon dioxide in the sample.

In this method, tubes *E*, *F* and *G* of Fig. 20 are omitted and the Meyer tube, Fig. 21, is substituted for *H* of Fig. 20.

Volumetric, by Absorption in Barium Hydroxide.—Three different methods of procedure are offered: (1) The gas is absorbed in a standard solution of barium hydroxide, the unused excess of the latter being titrated by standard hydrochloric acid without previous filtration. (2) The barium carbonate is removed by filtration before titrating the unused base. (3) The barium carbonate is filtered out, washed and dissolved in an excess of standard acid, the unused acid being then titrated by standard base. In method (3) a more concentrated solution of barium hydroxide may be used and it need not be standardized. Phenolphthalein is used as indicator in all three methods. Method (1) will be described.

Apparatus.—Parts *A*, *B*, *C*, *D* and *J* (including clamps *e*, *k* and *f*) of the apparatus shown in Fig. 20 are used. If hydrochloric acid is used for decomposing the carbonate, tube *E*, filled with pumice bearing silver sulphate in concentrated sulphuric acid, is required also. Drying tubes *F* and *G* are omitted. For the absorption the Meyer tube, shown in Fig. 21, is suitable.

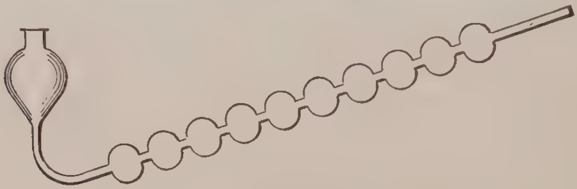

Fig. 21.—The Meyer absorption tube.

Barium Hydroxide Solution.—A saturated solution of barium hydroxide is prepared by warming and stirring the solid base with recently boiled water, using a ratio of 70 to 100 gm of base to 1000 cc of water, according to the purity of the barium hydroxide. Cool to room temperature and siphon into a bottle to be closed with a rubber stopper. Dilute 550 cc of this solution to 1000 cc with distilled water, mix and empty into a bottle which is provided with a siphon or similar outlet, from which the solution may be drawn, and a guard tube of soda lime to remove carbon dioxide from the air which is drawn in.

The last diluted solution should be about 0.25 normal. It is not adjusted to any exact normality because its concentration is subject to change with time. Instead, the standard acid to be prepared is taken as the primary standard and "blank" titrations are made frequently.

Sodium Carbonate for Standardizing Acid.—Prepare as directed on page 57.

Hydrochloric Acid.—From the known per cent of hydrochloric acid and the specific gravity of the concentrated solution in the laboratory, calculate the volume of solution necessary to make a suitable quantity (1200 cc to 10 liters, according to whether this is to be an individual preparation or laboratory stock) of solution, either fourth-normal or of such strength that 1 cc shall be equivalent to 0.005 gm of carbon dioxide, calculating equivalent weights from the equations:

$$CO_2 + H_2O \rightarrow H_2CO_3; \tag{1}$$

$$Ba(OH)_2 + H_2CO_3 \rightarrow BaCO_3 + 2H_2O; \tag{2}$$

$$Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O. \tag{3}$$

Measure 2 per cent more than this amount, using a graduated cylinder. Empty into a bottle of suitable capacity and add the necessary quantity of water. Stopper and mix thoroughly. Since the solution has been warmed by the reaction between acid and water it should stand until the temperature of the room is attained, before standardizing.

Standardization.—Calculate the weight of sodium carbonate required for 250 cc of a solution of strength equivalent to that of the desired acid. Weigh this quantity of the prepared pure material on counterpoised glasses and brush into a funnel which is placed in the neck of a 250-cc volumetric flask. Rinse down with distilled water, remove the funnel and dilute to the mark on the flask. Mix thoroughly, best by pouring into a dry beaker or flask and back several times.

Fill a burette with the solution and another with the acid solution already made and titrate as directed on page 58. Calculate the normality of the solution, also the volume of water to be added to each 1000 cc to make a solution of the exact concentration required. If water to be added is more than 10 cc add nearly the required amount to each liter of the acid, mix, and restandardize. If the quantity to be added is less than 10 cc the acid is diluted as follows: Fill a dry 1000-cc graduated flask to the mark with the acid solution. This flask should be capable of holding the required amount of water *above* the mark. From a burette add the calculated quantity of water directly to the solution in the flask and mix thoroughly. Pour into a dry bottle and make more diluted solution in the same manner, having first rinsed and dried the graduated flask. Check the accuracy of the dilution by restandardization.

A preliminary titration should be made to determine whether the barium hydroxide solution is approximately equivalent to the standard acid. Measure 25 cc of the base into an Erlenmeyer flask, add a drop of phenolphthalein and titrate rapidly to the disappearance of the pink color. Twenty-two to 27 cc of acid should be required, although no special note is made of the exact quantity, the value of the basic solution being determined accurately by a blank titration, made at the time the carbonate analysis is carried out.

Water, Free from Carbon Dioxide.—All water that is to be used for dilutions and for rinsing apparatus must be free from carbon dioxide and it must be neutral to phenolphthalein. Carbon dioxide is removed by boiling the water for 10 minutes, cooling and placing in a bottle provided with a guard tube filled with soda lime. Boiling large quantities of water is often not conveniently done in the laboratory. An equally satisfactory method is to provide the bottle with a two-hole stopper through which pass two glass tubes. One of these is for entering air. It is attached to a tube of soda lime outside and it extends to the bottom of the bottle. (The soda lime must be well confined by a filter of cotton.) The other tube is connected with an air pump. The purified air is then drawn through the water for an hour, after which the bottle is stoppered.

The three reagents may be placed on a shelf and connected as shown in Fig. 22, for convenient use.

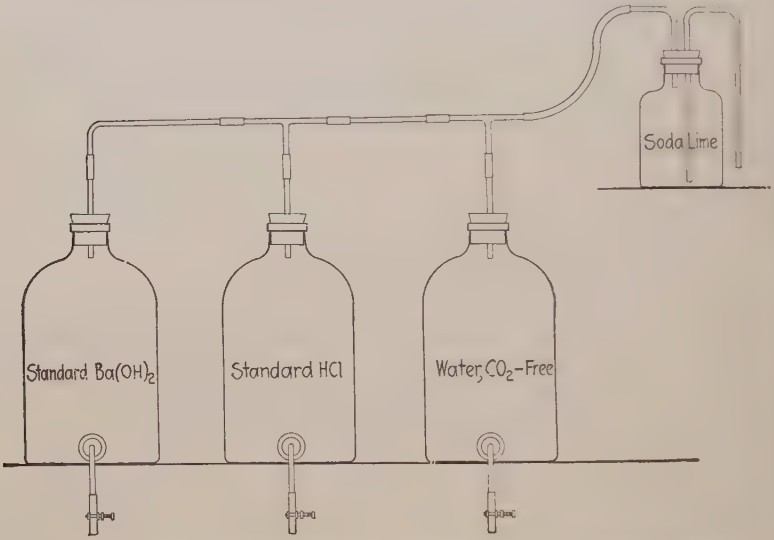

FIG. 22.—Assembly of reagents for the carbon dioxide determination.

Determination of the Carbonate Radical: *Volumetric Method.*—Assemble the apparatus discussed above, the Meyer absorption tube being half filled with distilled water. Close the cock of funnel *B* and test for leaks by opening cocks *k*, *f* and *e*. Water will flow from the aspirator until the pressure within the apparatus is reduced to equilibrium with the water column. If an air leak exists at any point between *B* and the Meyer tube air will bubble slowly through the tube. Any leak thus evidenced must be located and stopped.

The clamp at *k* is now closed and the cock of the dropping funnel *B* is opened. With *e* and *f* left open *k* is so adjusted as to allow about three bubbles of air per second to pass through the Meyer tube. Clamp *k* is not thereafter changed and this keeps the gas flow under control. Now close *f* and remove the flask *A*. Weigh into this about 0.3 gm of the sample of carbonate. One-half of the factor weight (pages 3 and 8) may be used, if desired. This would be 0.2750 gm for a fourth-normal solution, or 0.2500 gm for the solution of which 1 cc is equivalent to 0.005 gm of carbon dioxide. (The student should prove this statement.)

The flask is now replaced in the train and the dropping funnel and cocks *e* and *f* are opened. Two hundred cubic centimeters of water is allowed to flow from the aspirator, to remove carbon dioxide from the apparatus. The Meyer tube is now removed and emptied and 50 cc of barium hydroxide solution is measured into this tube from a burette or an automatic pipette, first discarding the few drops that are in the outlet of the measuring instrument. Add to the tube, from a graduated cylinder, enough cold carbon dioxide-free water to bring the water to the lower edge of the upper bulb when gas is flowing. The quantity of water necessary should be measured, once for all, so that it can be added without delay in subsequent determinations.

Replace the Meyer tube in the train and add 50 cc of dilute sulphuric or hydrochloric acid to the funnel *B*, the stopcock being closed. Now open *e* and *f*, then admit acid to the carbonate at a moderate rate until the entire 50 cc has entered. Finally close the stopcock and heat the acid slowly to boiling. Boil for 1 minute then remove the flame and open the stopcock. Pass air through until 1 liter of water has run from the aspirator, then close *e*. Remove the Meyer tube and rinse the contents into a 500-cc flask, using the carbon dioxide-free water but not from the ordinary wash bottle which is operated by blowing. Add a drop of phenolphthalein and titrate rapidly and with continuous stirring, to the disappearance of color.

While the experiment just described is under way, measure 50 cc of barium hydroxide into another flask, add approximately the same amount of carbon dioxide-free water that was used in diluting the solution for absorption and titrate with standard acid. From the volume of acid here required deduct that used for the base after the absorption of carbon dioxide. The remainder is the volume of standard acid equivalent to carbon dioxide absorbed. Calculate the per cent of carbon dioxide, or of the carbonate radical, in the sample of carbonate.

"Alkalinity" of Carbonates.—The methods that have just been described furnish a means for determining directly the actual carbon dioxide of carbonates. It is sometimes desirable to determine the power of a carbonate to neutralize acids. This can be calculated from the known carbon dioxide content only upon the assumption that no other basic substance is present. This assumption is not always correct. For instance, soda lime (essentially a mixture of sodium hydroxide and calcium oxide, but always containing some carbonate) has a much greater power for neutralizing acids than would be indicated by the carbon dioxide obtained from it. The same is true of lime that is partly air slaked, or of soda ash, which might contain hydroxide.

It is also true that the basic strength or "alkalinity" is often the figure that is desired and this may be obtained more quickly by a direct titration method. With carbonates that are soluble in water this is accomplished by dissolving a weighed sample, adding methyl orange and titrating with standard acid. If the carbonate is only slightly soluble in water an excess of standard acid is added. This dissolves the carbonate and the unused excess of standard acid is then titrated with a standard base. In this case, if the solution has been boiled to remove carbon dioxide, phenolphthalein or methyl red may be used as indicator but the same indicator must have been used when standardizing the base against the acid.

Soda Ash.—The standard acid that was used in the preceding exercise is suitable for this determination. The soda ash may be weighed on a counterpoised glass, if this is done quickly.

Determination of Alkalinity of Soda Ash.—Weigh about 2.5 gm of sample, dissolve in a small beaker and rinse the solution into a 250-cc volumetric flask. Dilute to the mark and mix well. By means of a pipette, measure 50 cc of the solution into a 250-cc Erlenmeyer flask or beaker, dilute with 50 cc of water, add a drop of methyl orange and titrate to the color change with standard acid. Calculate the per cent of sodium carbonate in the sample. This, of course, is upon the arbitrary assumption that no other carbonate or basic substance is present. Sometimes the alkalinity is expressed in terms of sodium oxide, Na_2O.

Limestone.—Powdered limestone is used for neutralizing the acidity of soils. If the alkalinity is calculated in terms of calcium carbonate the result may be greater than 100 per cent in case of dolomitic limestones, on account of the presence of magnesium carbonate, a substance of lower equivalent weight than that of calcium carbonate. Although a figure so obtained would be fictitious, in one sense, it is after all a practical basis for calculating the amount of stone required. If a determination of soil acidity should indicate n pounds of pure calcium carbonate required per acre, a sample showing by analysis a calculated per cent of 105 would be used in the ratio of $\frac{n}{105}$ pounds per acre, no matter what carbonates were actually present.

Determination of Alkalinity of Limestone.—Prepare a solution of sodium hydroxide, equivalent to the standard acid already on hand, or use the basic solution prepared for acidimetry, page 59. This should be made from material as nearly as possible free from carbonates. Sodium hydroxide purified by alcohol is best and the water should first be boiled, to expel carbon dioxide, then cooled. Standardize the solution by titrating against the standard acid, using methyl red or phenolphthalein. (See below.)

The sample should be ground to pass a 100-mesh sieve and it must be well mixed. Weigh 0.5 gm of the sample on counterpoised glasses and brush into a 250-cc Erlenmeyer flask. Moisten with water and then pipette 75 cc of the standard acid into the flask. After effervescence has nearly ceased connect the flask with a reflux condenser by means of a rubber stopper. Boil gently for 5 minutes to expel carbon dioxide and to insure complete solution of all carbonate, then cool. Rinse down the condenser, the stopper and the upper part of the flask. Add a drop of methyl red or of phenolphthalein and titrate the excess of acid by means of standard sodium hydroxide. (If phenolphthalein is used as indicator, the base must have been standardized by use of the same indicator. Also, in this case water that is used for rinsing must be free from carbon dioxide and it must not be blown in from the wash bottle.)

Calculate the alkalinity of the limestone in terms of per cent of calcium carbonate.

PHOSPHATES

Gravimetric, as Magnesium Pyrophosphate.—Any phosphate that is directly soluble in water can contain only metals of the alkali group, as all other phosphates have a relatively low degree of solubility. Solutions of phosphates or of phosphoric acid may be precipitated as dimagnesium ammonium phosphate by the addition of magnesium chloride or sulphate, the solution being made basic with ammonium hydroxide:

$$Na_3PO_4+MgCl_2+NH_4OH\rightarrow MgNH_4PO_4+2NaCl+NaOH; \quad (1)$$
$$Na_2HPO_4+MgCl_2+NH_4OH\rightarrow MgNH_4PO_4+2NaCl+H_2O; \quad (2)$$
$$H_3PO_4+MgCl_2+3NH_4OH\rightarrow MgNH_4PO_4+2NH_4Cl+3H_2O. \quad (3)$$

The precipitate always contains a variable amount of water of crystallization and it is therefore not weighed directly. Ignition converts it definitely into magnesium pyrophosphate:

$$2MgNH_4PO_4\rightarrow Mg_2P_2O_7+2NH_3+H_2O. \quad (4)$$

In practice, the concentration of ammonium hydroxide and of ammonium salts must be regulated within certain limits to prevent the partial precipitation of certain other phosphates which cannot be changed to pyrophosphate by ignition. For example, *mono*magnesium ammonium phosphate, formed to some extent if ammonium salts are present in immoderate quantities, decomposes at high temperatures into magnesium metaphosphate:

$$Mg[(NH_4)_2PO_4]_2 \rightarrow Mg(PO_3)_2 + 4NH_3 + 2H_2O. \qquad (5)$$

Also, *tri*magnesium phosphate, $Mg_3(PO_4)_2$, may be precipitated if the solution contains too much ammonia and this will remain unchanged by ignition.

Insoluble Phosphates.—Phosphates of other than alkali metals are usually soluble in acids but a direct precipitation of magnesium ammonium phosphate cannot be made because phosphates of the original metals reprecipitate as soon as ammonium hydroxide is added to neutralize the acid. For example, tricalcium phosphate, $Ca_3(PO_4)_2$, furnishes much of the phosphorus of fertilizers as the mineral apatite. This is soluble in acids but if an attempt were made to determine the phosphorus by a magnesium precipitation, the precipitate would be a mixture of phosphates of calcium and magnesium.

In order to prepare such a phosphate for a determination of phosphorus a preliminary separation of the phosphate radical is made by the addition of ammonium molybdate to the solution in nitric acid. This results in the formation of a yellow precipitate of ammonium phosphomolybdate:

$$(NH_4)_3PO_4 + 12(NH_4)_2MoO_4 + 24HNO_3 \rightarrow$$
$$(NH_4)_3PO_4.12MoO_3 + 24NH_4NO_3 + 12H_2O. \qquad (1)$$

This is filtered out and washed free from the alkaline earth metals. The precipitate is then dissolved in ammonium hydroxide, reforming ammonium molybdate and ammonium phosphate, both of which are soluble. This reaction is represented as follows:

$$(NH_4)_3PO_4{\cdot}12MoO_3 + 24NH_4OH \rightarrow (NH_4)_3PO_4 +$$
$$12(NH_4)_2MoO_4 + 24NH_4NO_3 + 12H_2O. \qquad (2)$$

The magnesium salt is now added and the precipitation and subsequent treatment are carried out as described for soluble phosphates.

Determination of Phosphorus in Soluble Phosphates.—Prepare a solution of "*magnesia mixture*" as follows: Dissolve 55 gm of crystallized magnesium chloride and 140 gm of ammonium chloride in water, add 130 cc of ammonium hydroxide (specific gravity 0.90) and dilute to 1000 cc. If this solution is kept in stock for any considerable time it will acquire a flocculent precipitate of hydrated silica, derived from solution of the glass by the base. The solution must be clear when used. This condition may be insured by filtering the solution or by preparing only enough of the reagent to last a short time.

Weigh duplicate samples of 0.2 to 0.4 gm of the phosphate into beakers of resistance glass, dissolve and dilute to 75 cc. Add a drop of methyl red and if a basic reaction is not shown add dilute ammonium hydroxide until the solution becomes yellow, avoiding an excess. Add 10 cc of a 10-per cent solution of ammonium chloride, mix and then add, very slowly, "magnesia mixture" sufficient in quantity to precipitate all of the phosphate. As the precipitate does not form rapidly in a barely basic solution it is not always easy to determine when enough of the reagent has been added. It is then best to use what is thought to be a good excess and to rely upon testing the filtrate which is obtained later.

Allow to stand for 15 minutes until a considerable part of the precipitate has appeared, then add concentrated ammonium hydroxide solution (specific gravity 0.90), in such quantity that the solution shall finally contain ammonium hydroxide equivalent to one-ninth of its total volume. Cover and allow to stand for 3 hours or stir continuously for 30 minutes. A small stirring machine may be used for this purpose.

Filter the precipitate on a filter of extracted paper, in a weighed platinum Gooch crucible or in an ignited and weighed alundum crucible, and wash until free from chlorides with a solution containing 2 per cent of ammonia or 10 per cent of ammonium nitrate, finally testing a few drops of the washings with silver nitrate after acidifying with nitric acid.

Set the filtrate aside, after adding 5 cc more of magnesia mixture. If more precipitate forms after standing an hour this must be filtered out, washed and added to the main portion.

If a Gooch crucible has been used for filtration, place the cap on the bottom and heat over the burner until dry, then over the blast lamp for 20 minutes. An alundum crucible is treated similarly. If a paper filter was used remove the paper from the funnel, fold and place in a weighed porcelain or platinum crucible. Incline the crucible with the cover leaned against it and heat gently over the burner until the paper is completely burned and the precipitate is nearly white. After the precipitate is white or light gray the crucible is heated for 20 minutes over the blast lamp, cooled in the desiccator and weighed. From the weight of magnesium pyrophosphate calculate the per cent of phosphorus, of phosphorus pentoxide or of the phosphate radical, according to the nature of the sample used.

Determination of Phosphorus in Rock Phosphate.—Prepare a solution of *ammonium molybdate* as follows:

Dissolve 100 gm of molybdic acid in a mixture of 145 cc of concentrated ammonium hydroxide (specific gravity 0.90) and 270 cc of water. Pour this solution slowly and with vigorous stirring into a mixture of 490 cc of concentrated nitric acid (specific gravity 1.42) and 1150 cc of water. Allow to stand at a temperature of 30 to 40° for several days, then decant and preserve in glass-stoppered bottles.

The phosphate sample should be finely ground and well mixed. Weigh about 2.5 gm, accurately to milligrams, and brush into a 250-cc flask. Add 30 cc of concentrated nitric acid and 5 cc of concentrated hydrochloric acid and warm until solution is complete, or until only insoluble silicious matter remains. Cool, rinse into a 250-cc volumetric flask, dilute to the mark and mix well. Pour the solution into a dry filter. Discard the first 10 to 25 cc of filtrate, and receive the remainder in a dry flask.

Pipette 25-cc portions of the clear solution into 250-cc Erlenmeyer flasks. Add ammonium hydroxide until a slight precipitate of hydroxides of iron, aluminium or other earth metals persists. Clear with a few drops of nitric acid, dilute to about 100 cc and heat to 60 to 65°. Immerse the flask in water which is at this temperature, a thermometer being placed in the flask. Add 75 cc of molybdate solution, mix and maintain at the temperature noted above for 1 hour. Filter and wash well with 10-per cent ammonium nitrate solution. The precipitate that adheres to the flask need not be removed but it must be well washed. Test the filtrate by adding more molybdate and returning to the water bath. If more precipitate forms it must be added to the main body.

Place the flask in which precipitation was made under the funnel and drop over the paper enough concentrated ammonium hydroxide to dissolve the precipitate, avoiding unnecessary excess. Wash this solution into the flask below with hot water and, if necessary, add more ammonium hydroxide to dissolve all of the precipitate in the flask. Wash the paper thoroughly with hot water, then rinse the entire solution into a 200-cc beaker and cool to room temperature. Nearly neutralize with hydrochloric acid, leaving the solution slightly basic. The formation of yellow precipitate and its subsequent resolution by ammonia is sufficient indication.

Cool if necessary and add, very slowly and with vigorous stirring, 15 cc of magnesia mixture. After 15 minutes add concentrated ammonium hydroxide as directed for analysis of soluble phosphates, page 89, and proceed from this point as there directed. Calculate in the same way.

Volumetric, by Titration of Ammonium Phosphomolybdate.—It has been shown that the precipitation of ammonium phosphomolybdate from acid solutions of phosphates provides a means for separation from metals that would form insoluble phosphates in basic solutions. This precipitation also serves as a basis for an *indirect* determination of phosphorus. The formula already given for the double compound shows that it is regarded as a compound of ammonium phosphate and molybdenum trioxide, the latter being the anhydride of molybdic acid. It is therefore capable of neutralizing a base, as was shown in Eq.

(2) on page 88. If the base is added as a standard solution in measured excess and the unused portion is titrated by a standard acid the phosphorus (or the corresponding phosphoric anhydride) may be calculated.

Variation in Composition.—It has been found that the ratio of molybdic anhydride to ammonium phosphate in the precipitate is somewhat variable unless the conditions of precipitation are standardized and kept constant. This is probably due to the coprecipitation of some free molybdic acid. Such variations must occasion an error in the volumetric determination, since it is the molybdic anhydride or acid that furnishes the acid properties of the compound. If the method is followed as outlined the composition of the precipitate will be fairly accurately represented by the formula already given.

Titration.—Sodium hydroxide reacts as follows:

$$(NH_4)_3PO_4.12MoO_3 + 24NaOH \rightarrow (NH_4)_3PO_4 + 12Na_2MoO_3 + 12H_2O. \quad (1)$$

Upon addition of acid any excess of base is neutralized and if the acid is added to a color change with phenolphthalein, triammonium phosphate will have been changed to diammonium phosphate, since the normal salt is basic to this indicator. The net result is therefore to be expressed by the following equation:

$$2(NH_4)_3PO_4.12MoO_3 + 46NaOH \rightarrow 2(NH_4)_2HPO_4 + 23Na_2MoO_3 + (NH_4)_2MoO_4 + 22H_2O. \quad (2)$$

It should be noted that it is necessary to have the solution cold when the excess of base is added and to have present sufficient water to prevent the escape of ammonia, which would be produced by reaction of ammonium phosphate with sodium hydroxide.

Determination of Phosphorus in Rock Phosphate: *Volumetric Method.*—The molybdate solution that was used for the gravimetric determination may be used here also, first adding 5 cc of concentrated nitric acid to each 100 cc of solution. This additional acid serves to prevent the precipitation of molybdic acid.

Prepare a half-normal solution of hydrochloric acid, standardizing against sodium carbonate as directed on page 83, modifying the weights according to the different normality of this solution. Prepare a half-normal solution of sodium hydroxide in boiled and cooled water, standardizing against the acid, using phenolphthalein.

Use 2.5 gm of sample, weighed accurately to milligrams. Dissolve as directed for the gravimetric determination of phosphorus in rock phosphate, page 90, and dilute the solution to 500 cc in a volumetric flask. Mix well and pour into a dry filter. Reject the first 25 cc and collect the rest in a dry flask. Pipette 25-cc portions of this solution into 250-cc flasks. Add ammonium hydroxide until a slight precipitate persists and clear with a few drops of nitric acid. Place a thermometer in the flask, immerse in the water bath and heat to 65°, then add 35 cc of freshly filtered molybdate solution. Mix and leave the flask in the water bath for 15 minutes, then filter at once. Wash twice with water by decantation, using 25 cc each time, pouring the washings into the filter. Transfer the precipitate to the filter as thoroughly as can be done without the use of a policeman and wash the flask and filter with cold water until the filtrate from two fillings of the filter yields a pink color upon the addition of phenolphthalein and one drop of standard base. (Test the wash water in this manner before using.)

Return the filter and precipitate to the flask in which precipitation was made and add 50 cc of *cold* water. Add half-normal base from a burette until all of the precipitate is dissolved, mixing by gentle rotation. Immediately add a drop of phenolphthalein and titrate with standard acid. From the total volume of base used, deduct that equivalent to the acid required and from the remainder calculate the per cent of phosphorus and of phosphorus pentoxide in the sample.

PART II

SPECIAL MEASUREMENTS

In addition to the work of a strictly analytical nature the quantitative laboratory is usually equipped for certain quantitative measurements which involve the use of special instruments, not mentioned or described in the preceding pages. In this division certain of these instruments will be described and a brief discussion of theoretical principles will be given, together with directions for making the measurements. The application of the results will be discussed also. Later (in the work of Part III) these instruments will be used for testing agricultural materials.

CHAPTER IV

DENSITY AND SPECIFIC GRAVITY

Density.—The density of a given substance is the mass of unit volume. When the metric system of weights and measures is used, as is customary in most scientific work, the density equals the weight, in grams, of 1 cc of substance.

Specific Gravity.—Specific gravity is the ratio of the density of a given substance to that of some other arbitrarily chosen substance. For liquids and solids the substance which is generally chosen for reference is water. Since the weight of a milliliter (which, for all practical purposes, may be taken as a cubic centimeter) of water at 4° is, by definition, 1 gm, the density of any substance becomes its specific gravity if the latter is referred to water at 4°. This is the preferred method for expressing specific gravity and the figure so determined is stated as

$$\text{specific gravity}^{\overset{t^\circ}{4^\circ}}$$

for the specific gravity when the substance is at temperature t°.

Most of the laboratory methods for determining specific gravity involve measuring either (*a*) the buoyant effect of the (liquid) substance upon an immersed "float," (*b*) the comparative weights of equal, but unmeasured, volumes of the (liquid) substance and water, or (*c*) the weight of water displaced by a weighed, but not measured, quantity of the (solid) substance. To carry out such experiments at 4° is a problem offering great experimental difficulties and the work is usually performed at some more convenient temperature, higher than 4°. On this account it is customary to express the results as

$$\text{specific gravity}^{\frac{t^\circ}{t^\circ}}$$

this quantity being the ratio of the density of the substance at t° to that of water at the same temperature. This quantity can be converted into the specific gravity at $\frac{t^\circ}{4^\circ}$ by multiplying by the density of water at $t°$. Or it can be converted into specific gravity at $\frac{t°}{t_1°}$ by multiplying by the ratio of the density of water at $t°$ to that at $t_1°$, the latter symbol representing any desired temperature.

It cannot be too strongly emphasized that both temperatures represented in these symbols should always be expressed or understood. Because of failure to do this there is much confusion in the records in scientific literature.

Baumé System.—In this system two scales are used, one being for liquids lighter than water, the other for liquids heavier than water. The first is applicable to petroleum products and to most other oils and fats. The second scale is used for most solutions in water.

In the original Baumé scale for liquids heavier than water the point to which a hydrometer float sinks in a solution of sodium chloride, 15 per cent by weight and at 15° C., was taken as 15° Baumé (abbreviated Bé.). The corresponding point for pure water was taken as 0° Bé. and all other points were located by these two. For liquids lighter than water the scale has the point 10° Bé. for pure water and 0° Bé. for a 10-per cent solution of sodium chloride, the scale being extended beyond 10° for lighter liquids. It will be seen that this is a wholly arbitrary system and conversion of degrees Baumé into specific gravity, or vice versa, will involve the use of special formulas. Several modifications of the original Baumé scales have come into use and the difficulties involved in interpretation have been correspondingly increased. On account of the complexity of the system and the fact that such a system seems quite unnecessary it is unfortunate that it has become so generally used in the chemical industries.

No one set of formulas can serve for the conversion of specific gravity and Baumé degrees into one another but as the system is at present used in many industrial laboratories the following formulas will be useful.

For liquids heavier than water:

$$S = \frac{145}{145 - B}$$

and

$$B = 145 - \frac{145}{S}.$$

For liquids lighter than water:

$$S = \frac{140}{130 + B}$$

and

$$B = \frac{140}{S} - 130,$$

where B = degrees Baumé and S = specific gravity at 15.5° C.

Methods for Determining Specific Gravity. The Picnometer. The most accurate method for making this determination depends upon the use of a vessel known as a "picnometer." There are various forms of picnometers but the instrument is essentially a small flask which may be weighed, first filled with water and then with a liquid whose specific gravity is to be measured. Specific gravity at $\frac{t°}{t°}$ is given by the ratio of the two weights, as explained above. Two forms of picnometers are shown in Figs. 23 and 24.

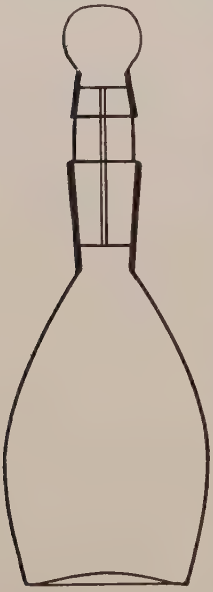

Fig. 23.—Picnometer bottle, with cap.

The picnometer flask has an accurately ground stopper which is bored longitudinally as a capillary tube. The dry flask is first weighed. Filled with distilled water, which has been boiled recently to expel dissolved air, it is then nearly immersed in a constant temperature bath and when the water has been brought to the required temperature the surplus drop is removed from the top of the stopper. The flask is then removed from the bath, wiped dry and weighed. It is necessary that the room temperature shall be a few degrees lower than that of the bath, so that the liquid may recede from the tip of the stopper after removing from the bath.

After the weight of water contained at $t°$ has been determined the flask is emptied, rinsed with redistilled alcohol and dried. It is then filled with the liquid whose specific gravity is to be measured and this is treated in the same way as was the water.

The weight of this liquid divided by the weight of water gives the specific gravity at $\frac{t^\circ}{t^\circ}$.

A modified Ostwald picnometer is shown in Fig. 24. For filling, a rubber tube is attached to the larger branch of the picnometer. By dipping the other end into a liquid and applying suction to the rubber tube the picnometer is filled with liquid. It is then hung in a constant temperature water bath. After the liquid has reached the temperature of the bath the liquid is carefully blown out until it stands at the mark on the tube. After taking off the surplus drop from the upper end the liquid is allowed to fall back into the picnometer. The latter is then removed from the bath, the rubber tube is removed and the instrument is wiped dry and weighed. The calculations are the same as with the flask form of picnometer.

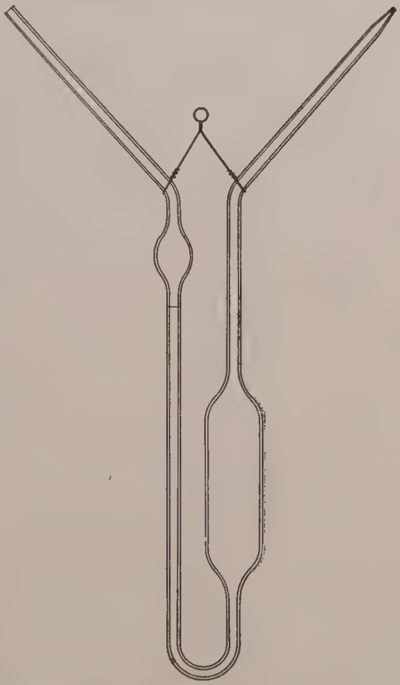

FIG. 24.—Picnometer, pipette form.

Hydrometer.—The floating hydrometer (Fig. 25) or "spindle" is a bulb, weighted at the bottom and having a slender upper stem which will be partly immersed when the hydrometer is floating in a liquid. The depth to which the instrument will sink depends upon its displacement of liquid which, with a given instrument, depends in turn upon the density of the liquid. A scale upon the stem provides the necessary means for making the observation.

The graduations on the hydrometer stem may indicate either specific gravity or Baumé degrees. Also there are numerous special hydrometers, reading in various scales

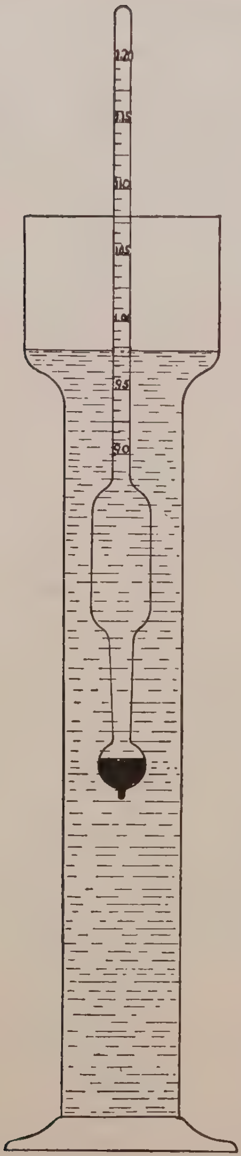

Fig. 25.—Floating hydrometer and cylinder.

which apply to special industrial uses. Two of these will be mentioned.

Lactometer.—This is an instrument much used in dairy laboratories. Quevenne's lactometer is graduated in degrees from 15 to 40, corresponding to specific gravity 1.015 to 1.040. The New York Board of Health lactometer also is graduated in arbitrary degrees in such manner that 0° corresponds to specific gravity 1.000 and 100° to 1.029, the latter figure being considered as the average specific gravity of pure milk. Degrees on this lactometer would thus roughly indicate the per cent of whole milk in a milk and water mixture.

Other special names, such as "saccharometer," "alcoholometer," etc., apply to hydrometers for sugar solutions, alcohol and other special uses.

In using hydrometers the temperature at which the experiment is performed must be that for which the instrument is calibrated and care must be taken to remove air bubbles which might cling to the hydrometer and thus increase the effective displacement.

The floating hydrometer is much used for measurements not requiring great accuracy, as a reading is very quickly made. The scales found on the stems are frequently very inaccurate and any hydrometer should be calibrated by the use of liquids of known specific gravity.

Westphal Balance.—In effect, this balance (Fig. 26) weighs the liquid which is displaced by an immersed plummet whose displacement of water is known. The balance is first brought into adjustment with the dry plummet hanging on the beam. A cylinder containing the liquid is then brought under the beam in such a position as to allow the plummet to be totally immersed in the liquid. Weights are then placed on the beam to bring the balance again into adjustment. These weights are so related to the volume of the plummet and to the graduations on the beam as to give directly the specific gravity at $\frac{t^\circ}{t^\circ}$. Usually the displacement of the plummet in pure water at the rated temperature is 5 gm and the figure on the beam directly over the plummet is 10. Therefore the largest weight piece should weigh 5 gm.

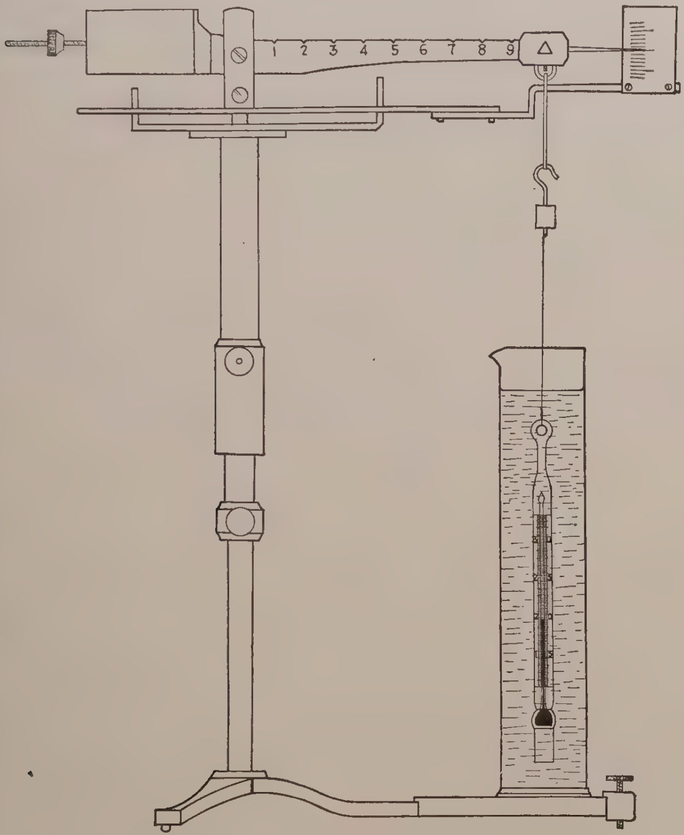

FIG. 26.—Westphal specific gravity balance.

In pure water this would be placed at 10 to bring the balance into equilibrium. This would read 10 tenths, or 1. Three other denominations of weights are provided, reading to the second, third and fourth decimal places, respectively.

Calibration.—This instrument, like the floating hydrometer, must be used at the temperature for which its displacement of water is known. Any plummet may be calibrated for use at its rated temperature or at any other desired temperature by weighing it dry, and again suspended in distilled water which has been boiled to expel dissolved gases, then cooled to the desired temperature. The difference between these weights represents the weight of water displaced. If the displacement so found is not exactly 5 gm, all specific gravity determinations are corrected to take account of the deviation, thus:

$$S^{\frac{t^\circ}{t^\circ}} = S_1{}^{\frac{t^\circ}{t^\circ}} \times \frac{5}{d}$$

where $S^{\frac{t^\circ}{t^\circ}}$ is the true specific gravity, $S_1{}^{\frac{t^\circ}{t^\circ}}$ the figure found experimentally and d the water displacement at t°.

The weights for the Westphal balance are calibrated by the method described for analytical weights, page 41.

Use of the Westphal Plummet on an Analytical Balance.—The Westphal balance is a convenient and low priced piece of apparatus for making specific gravity determinations with a fair degree of accuracy. Determinations may be made with a higher degree of refinement by using the Westphal plummet with a good analytical balance. The plummet is cleaned and dried, then suspended from a hook on the left arm of the balance. Weights are added to the right pan to counterpoise, then a cylinder of the liquid to be tested is placed on a bridge over the balance pan in such a way as to allow the balance to swing unimpeded, but supporting the cylinder so that the plummet is entirely immersed in the liquid. A counterpoise is again effected by removing weights from the right pan. The difference between the two weights is the liquid displacement. This divided by the water displacement, found as already described, gives the specific gravity at $\frac{t^\circ}{t^\circ}$. The mechanical arrangement is shown in Fig. 27.

Applications.—For the application of specific gravity determinations to the quantitative analysis of solutions, two conditions are necessary: The solution must be a binary one (having only two components, solvent and solute) and the variation of specific gravity with concentration must be great enough to make possible calculations of concentration to a reasonable degree of accuracy. Accurate tables have been worked out in a limited number of cases and for these the specific gravity determination frequently offers the most convenient method for the analysis. Examples of such cases are solutions of ethyl alcohol, methyl alcohol, various sugars and various acids in water. If it is known that only one of these compounds is present in a solution, the concentration can readily be determined. Of course a good thermostat is necessary, in order to avoid temperature errors.

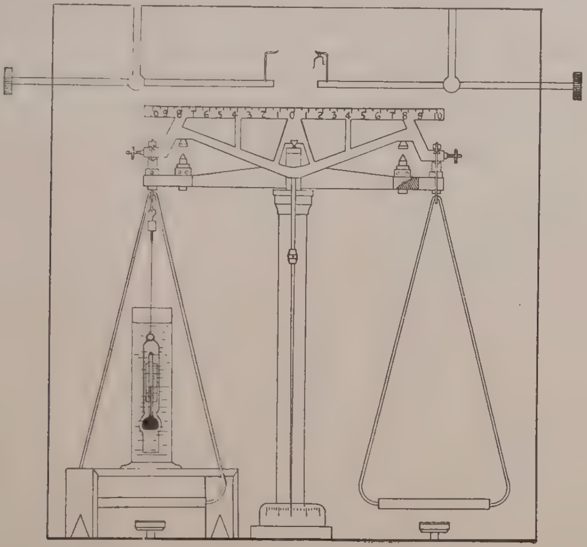

FIG. 27.—Westphal plummet as used with an analytical balance.

Such tables as those mentioned above are found in many of the standard handbooks. Quite elaborate tables are contained in "Methods of Analysis," published by the Association of Official Agricultural Chemists, and in U. S. Bureau of Standards Circular 19. The latter may be obtained from the Superintendent of Documents at small cost.

Determination of Specific Gravity.—Make accurate determinations of specific gravity of solutions of methyl alcohol, ethyl alcohol, cane sugar, etc., as they may be furnished by the instructor, and using the methods described in the foregoing pages. Report the per cent concentration of the solutions, found by reference to tables such as those mentioned above. The temperature used for the determination must correspond to that for which the table in question is constructed.

In Chap. X this determination is applied to the identification of the various oils, fats and waxes and in Chap. XI, dealing with the analysis of dairy products, the application of specific gravity determinations to milk testing is discussed.

CHAPTER V

HEAT OF COMBUSTION (CALORIMETRY)

All chemical reactions involve either evolution or absorption of heat energy. The measurement of the "heat of reaction" has proved to be a valuable method for investigation, in both pure and applied chemistry. We are here particularly concerned with the amount of heat evolved by the combustion of fuels and of foods. In the case of fuels this, of course, has a direct bearing upon the value of the fuel when it is burned for the production of useful heat, while the heat of combustion of foods and feeding stuffs is of interest in connection with their relative values as energy producers in the animal body. However, the student is cautioned against the fallacy that the "calorific value" (heat of combustion) of a food is the only criterion as to its value. Even an elementary study of physiology should convince one that such matters as balancing of diet, proper proportioning of rougher and more refined foods, stimulation of appetite, per cent of contained nitrogen, etc., are of prime importance in this connection.

Units of Measurement.—In scientific work the accepted unit of heat is the *calorie*. As ordinarily used this is the heat that is absorbed by 1 gm of pure water as its temperature rises 1° C. (strictly, from 15° to 16°). The specific heat of water is not the same for all temperatures but the variation is only 0.013 over a range of 0° to 100°.

In engineering work, for expressing the value of fuels, the *British thermal unit* (B.t.u.) is more often used. This is the heat absorbed by 1 lb of water as its temperature rises 1° F.

The "calorific value," "fuel value" or "heat units," as it is variously expressed, is the number of calories per gram or of B.t.u. per pound, made available by burning the material. The following equations represent relative values:

$$\text{cal per gm} \times 1.8 = \text{B.t.u. per lb;}^{1} \qquad (1)$$

$$\frac{\text{B.t.u. per lb}}{1.8} = \text{cal. per gm.} \qquad (2)$$

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[1] For the derivation of these formulas see Mahin, "Quantitative Analysis," 2nd ed., p. 314.

Apparatus.—A great many forms of calorimeters have been used for measuring heats of combustion but all of the more successful of these are based upon a measurement of the rise in temperature produced by burning a weighed sample in oxygen at high pressure, in such a way as to have the evolved heat absorbed by a known quantity of water and by the material of the

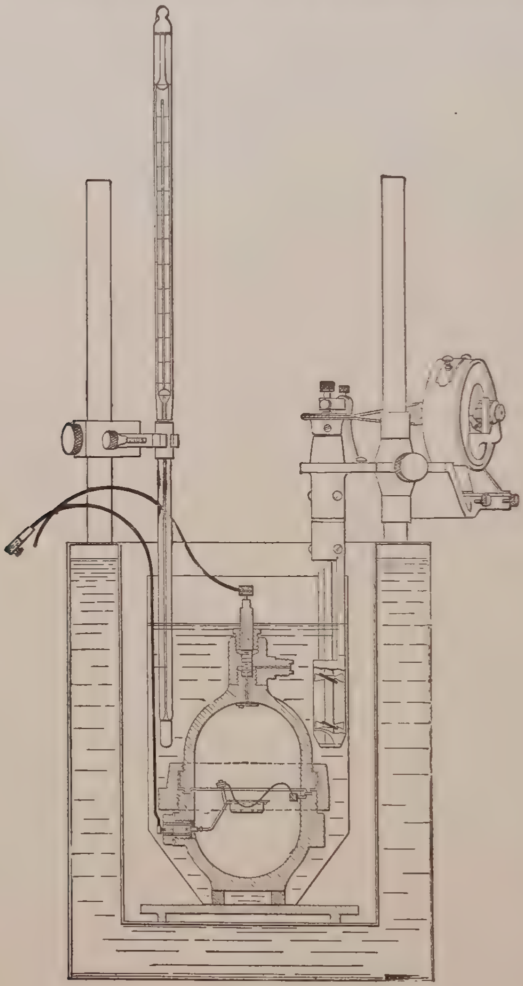

Fig. 28.—Section of the Emerson calorimeter.

calorimeter itself. The weights of these (and the known specific heat of the materials of the calorimeter), taken with the weight of the substance burned and the temperature rise, furnish the necessary data for the calculation.

Emerson Fuel Calorimeter.—Following is a description of the Emerson calorimeter and also directions for making the determination of fuel value.

Bomb.—The bomb, made of steel, consists of two cups joined by means of a heavy steel nut. The two cups are machined at their contact faces with a tongue and groove, the joint being made tight by means of a lead gasket inserted in the groove. The lining is of sheet nickel, platinum or gold, spun in to fit. The bomb is closed by a milled wrench or spanner. The pan holding the combustible is of platinum or nickel, and the supporting wire of nickel. (See Fig. 28.)

Calorimeter.—The jacket is a double walled copper tank, the space between the walls being filled with water. The calorimeter can is made as light as is possible, of sheet brass, nickel plated.

Stirring Device.—The stirrer is directly connected to a small motor and it is enclosed in a tube to facilitate its action in circulating the water. The stirrer is mounted on a post on the calorimeter jacket as is also the thermometer holder.

Ignition Wire.—Unless ignition of the fuel requires a very high temperature a platinum resistance wire is suitable. For ignition of such substances as are used in determining the water equivalent of the calorimeter (naphthalene or cane sugar) or of anthracite coal an iron wire is more certain in its action because it burns and produces a higher temperature. When iron wire is used a correction of 1600 calories per gram of wire is subtracted from the total calories obtained from the fuel combustion. This is the heat of oxidation of the iron.

Formation of Nitric Acid.—When any nitrogenous organic matter is burned in air practically all of the nitrogen is liberated in the elementary form. On account of the high concentration of oxygen in the calorimeter bomb a considerable portion of the nitrogen is oxidized and the products dissolve in the water which is formed by the combustion of hydrogen. A dilute solution of nitric acid is thereby formed. This gives rise to a positive error in the observation of fuel value, the magnitude of the error depending upon the extent to which nitric acid is formed. As a rule the error is small and it may be ignored for ordinary fuel testing but if a correction is to be made the nitric acid is titrated by standard base, at the end of the experiment.

The heat of formation and solution of nitric acid from elementary nitrogen is 230 calories per gram. It is convenient to use a standard solution of base, 1 cc of which is equivalent to 5 calories. The normality of such a solution is

$$\frac{5}{230 \times 0.06302} = 0.3450 \text{ N.}$$

The number of cubic centimeters of base required to titrate the nitric acid in the bomb after the combustion is multiplied by 5, the product being subtracted from the observed calories.

Radiation.—Radiation or absorption of heat by the calorimeter may be avoided by making the calorimeter "adiabatic." This may be done in a number of ways, three of which will be mentioned.

1. The water in the surrounding jacket may be heated by electrical means, so as to keep pace with the rise in temperature of the calorimeter water. This is the most satisfactory method, although somewhat complicated and expensive apparatus is required.

2. The water in the jacket may be warmed by chemical action. By Richards' method a basic solution is used to fill the jacket and an acid is run in from a burette at a rate which depends upon the rate of change in temperature of the calorimeter water and upon the concentration of the acid. The acid solution may be standardized in terms of the number of calories liberated by the action of each cubic centimeter upon the base, in which case the proper rate of addition is more easily determined.

3. The jacket of the calorimeter may be evacuated, on the principle of the Dewar flask, the transfer of heat outwardly then being limited to that which occurs through conductivity of the glass of the jacket. This would appear to be the least troublesome method but it has not worked well in practice.

Radiation Corrections.—If adiabatic conditions cannot be maintained several methods for making radiation corrections are available.

1. The combustion may be begun as far below atmospheric temperature as it is to end above it. By this means absorption of heat in the first half of the experiment would appear to balance radiation during the last half.

This is the roughest sort of approximation and it would not serve for ordinarily accurate work.

2. The rate of change of temperature may be observed for a certain period before firing and for another period after the calorimeter water has absorbed all of the heat from the bomb. The *average* of these rates is then considered to be the mean rate of absorption or radiation of heat for the entire experiment and if this is multiplied by the time elapsing between the firing and the maximum absorption the net gain or loss during the entire observation period is given.

This method is very commonly employed and it gives a very close approximation to the true correction.

3. Observations are made in the same way as in method (2). In addition the time, a, required for six-tenths of the total rise in temperature is observed, also the time, b, for the remaining rise. Instead of averaging the two radiation (or absorption) rates the preliminary rate, R_1, is multiplied by a and the final rate, R_2, by b. The corrected rise is then

$$T + R_1a + R_2b,$$

where $T =$ total rise, and R_1 and R_2 are regarded as positive for falling temperatures and negative for rising temperatures.

The observation of the time, a, is subject to some uncertainty when the temperature is rising rapidly and on this account the method is not so easily applied as is method (2). It will rarely be found that the difference between the corrections calculated by these two methods will differ by more than 0.2 per cent and as this is well within the permissible variation, method (2) is recommended for all but the most refined work.

4. The Regnault-Pfaundler method approaches theoretical accuracy more nearly than any of the methods already described. For a discussion of this method, see White, "Gas and Fuel Analysis" (International Chemical Series) 2nd ed., page 268.

Time-temperature Curves.—Three types of time-temperature curves are produced, according to whether the experiment is (*a*) begun and finished below room temperature, (*b*) begun below and finished above or (*c*) begun and finished above. These types are illustrated in Fig. 29. The relative slopes of the ends of the curves represent R_1 and R_2.

It will be observed that these slopes are easily determined in curve (*b*) but that it is especially difficult to decide as to what temperature should be taken as the maximum produced by the fuel combustion, in the experiment represented by curve (*a*). Conditions represented by curve (*b*) are to be obtained when possible.

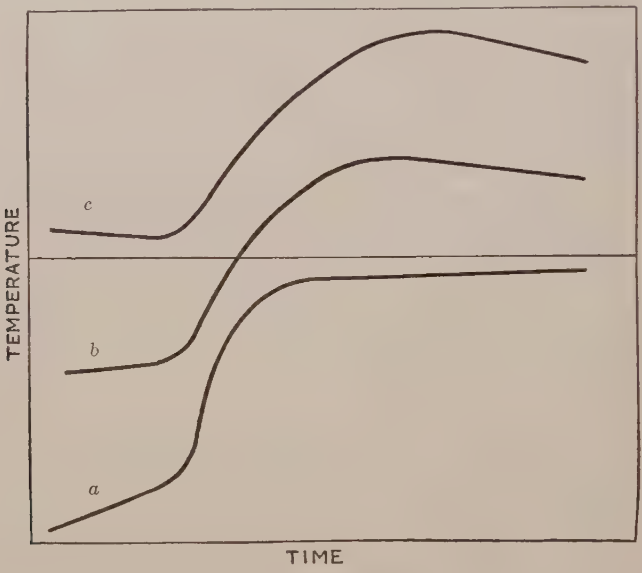

Fig. 29.—Time-temperature curves.

Determination of Heat of Combustion of Fuels, Foods or Feeds.—Place the lower half of the bomb in the holder and the fuel pan in the wire support, after having wired the fuse wire according to Fig. 28.

Extend the wire across the pan, allowing it to dip sufficiently to be in contact with the substance, which is later to be placed in the pan. The wire must in no case touch the pan. The fuse wire should be placed in series with two 100-watt lamps in parallel when the 110-volt power circuit is used for firing.

The material whose calorific value is to be determined should be ground to pass a 60-mesh sieve and it should be dried at 100° before weighing the sample for combustion. If the material is a liquid, such as milk, or a substance containing a large amount of water, 100 gm or more is first weighed (to centigrams only). It is then evaporated to dryness over the steam bath and reweighed. The loss of water gives the necessary data for calculating the fuel value of the dry material to a basis of the original sample. Thus, if M represents the per cent of water, c the calorific value of the dry residue and C that of the original sample,

$$C = \frac{(100 - M)c}{100}.$$

As suitable materials for exercises in calorimetry of foods, such substances as dried egg albumen, starch, sugar and butter fat may be used. Coal, coke, crude oil, kerosene or gasoline are fuels whose calorific power may be determined. Volatile liquids, such as the last named two, can be weighed and burned in a gelatine capsule, such as are used for medical preparations. Blank determinations must then be run on other similar capsules, so that corrections may be subtracted. All of the capsules are weighed.

Fill a weighing bottle with the prepared sample and weigh accurately to 0.1 mg. Pour from this into the pan in the bomb, until the pan is approximately half full. Weigh the bottle again, the difference between the above weighings giving the weight of the fuel in the bomb. This weight should be greater than 0.5 gm and not more than 1.2 gm. For hard coal the charge should be not greater than 1 gm. Hard coal should not be as finely divided as soft coal or foods.

The upper half of the bomb is now placed in position and the nut is screwed down as far as may be by hand, care being taken not to cross the threads. The shoulder on the upper half of the bomb, over which the nut makes bearing contact, should be lubricated with oil. Extreme care should be taken that no oil or grease is deposited on the lead gasket.

The bomb is now ready to be filled with oxygen. The nipple is coupled to the oxygen piping by means of the attached hand union. In handling the bomb, care should be taken not to tip or jar it, as fuel may be thrown from the pan.

The spindle valve on the bomb is opened one turn and then the valve on the oxygen supply tank is very cautiously opened. The pressure gauge should be carefully watched and the tank valve so regulated that the pressure in the system shall rise very gradually. When the pressure reaches 300 lb per square inch, the tank valve is closed and the spindle valve immediately afterward. The bomb should be immersed in water immediately to detect any possible leaks. The bomb is now ready for the calorimeter, which is prepared as follows:

Nineteen hundred grams of distilled water, weighed or measured in a calibrated flask, is placed in the calorimeter can at a temperature about 1.5° below the jacket temperature (which should be in the proximity of the room temperature). The bomb is then placed in the calorimeter and the stirrer and thermometer are lowered into position as indicated by Fig. 28. The thermometer is immersed about 3 inches in the water. The bulb of the thermometer should not touch the bomb.

The terminals of the electric circuit used for firing are now attached. Care should be taken that neither the bomb nor the stirrer is allowed to touch the sides of the can. The stirrer is now started and allowed to run 3 or 4 minutes to equalize the temperature throughout the calorimeter.

Readings of the thermometer are now taken for 5 minutes (reading to 0.001° or 0.002° every minute) at the end of which time the switch is turned on for an instant only, which will be found sufficient to fire the charge. In course of a few seconds the temperature begins to rise rapidly and approximate readings are taken every minute until the rise becomes slow, more accurate readings then being taken. After a maximum temperature is reached and the rate of change of temperature is evidently due only to radiation to or from the calorimeter, the readings are continued for an additional 5 minutes, reading every minute. These readings, before the firings and after the maximum temperatures, are necessary in the computation of the cooling correction. The time elapsed from the time of firing to the maximum temperature should be, in no case, more than 6 minutes.

When through with the run, replace the bomb in the holder and allow the products of combustion to escape through the valve at the top of the bomb. Unscrew the large nut and clean the interior of the bomb. The inside of the nut should be kept greased, also the threaded part at the top of the lower cup.

Immediately after each run, the lining of the bomb should be washed out with a cloth moistened with a dilute solution of ammonium hydroxide and then with water. When the apparatus, after using, is to be left for several hours or more before making another test, the linings should be removed and the inner surface of the bomb slightly coated with oil. This oil under the linings should be removed when next preparing the bomb for use, as an excess of it may be ignited with a possible resulting injury to the linings.

Heavy Oils, Coke and Hard Coal.—The determination of the heat of combustion of heavy oils, such as crude petroleum, and also of coke and extremely hard coals, is best made by mixing with a ready burning combustible, such as a high-grade bituminous coal or pure carbon. This auxiliary combustible facilitates the complete combustion of the whole mixture in the case of coke and hard coal, and with the heavy oil it acts as a holder and prevents rapid evaporation of the oil. The auxiliary combustible should be placed at the bottom of the pan and the coke, coal or oil sprinkled over it. It should be dried with extreme care and carefully standardized as to the resulting rise in temperature per gram in the calorimeter when completely burned.

Calculation.—First plot a smooth curve, using temperatures as ordinates and time as abscissas. Use only the straight portions of the ends of the graph for calculating R_1 and R_2.

The difference between the temperature at maximum and the temperature at firing gives directly the total rise in temperature in the calorimeter. To this rise a cooling correction must be applied, which is computed as follows:

The change in temperature during the preliminary 5 minutes of reading, divided by the time (5 minutes) gives the rate of change of temperature per minute, due to radiation to or from the calorimeter, and also any heating due to stirring. This factor is R_1 and in like manner the readings taken after the temperature change has become uniform give R_2. The two rates of change of temperature give the existing conditions in the calorimeter at the start and at the finish of the run. The algebraic signs of R_1 and R_2 will be $(+)$ for falling temperatures and $(-)$ for rising temperatures. Therefore, the algebraic sum of the two rates, divided by 2, will give the mean rate of change of temperature due to radiation or absorption, during the entire experiment. This value, multiplied by the time in minutes elapsing between firing and the attainment of maximum temperature, gives the total radiation correction. This radiation correction is thus expressed:

$$C = \frac{(R_1 + R_2)t}{2}, \tag{1}$$

where C = radiation correction,

t = time from firing to maximum

and R_1 and R_2 have the significance already explained. The radiation correction may have either a positive or a negative sign, according to whether the net effect was actual radiation or absorption of heat from the surrounding atmosphere.

The quantity C is applied to the total observed rise to obtain the corrected rise and the latter, divided by the weight of material burned, will give the rise per gram of sample. This rise per gram is multiplied by the weight of water used plus the "water equivalent" of the calorimeter and the product is calories per gram of sample. This figure multiplied by 1.8 gives B.t.u. per pound of sample, if this method of expression is desired.

Summarizing, if T = total observed temperature rise and e = water equivalent,

$$\frac{(T + C)(1900 + e)}{S} = \text{cal per gm} \tag{2}$$

Substituting the value of C,

$$\frac{\left(T + \frac{(R_1 + R_2)t}{2}\right)(1900 + e)}{S} = \text{cal per gm.} \qquad (3)$$

The water equivalent may be calculated from the known weights and specific heats of all of the parts of the calorimeter but it is better determined experimentally by burning a pure substance of known heat of combustion, such as naphthalene or cane sugar.

CHAPTER VI

INDEX OF REFRACTION

Theory.—When a ray of light passes from one transparent medium into another of different density, at the surface of separation the ray is always bent from its first course, unless it strikes this surface at an angle of 90°. This phenomenon is known as optical "refraction." The angle i, included between the incident ray and the normal to the separating surface (see Fig. 30) is the *angle of incidence* while the angle r, between the refracted ray and the normal, is the *angle of refraction.* The ratio $\frac{\sin i}{\sin r}$ is the *index of refraction.*

If s and s' represent, respectively, the speed of light in the medium from which the light emerges and in that into which it passes, then the index of refraction of the latter medium with respect to the former is greater than 1 if s is greater than s', and conversely. In a general way the speed of light varies inversely with the density of the medium, although this is not a strict mathematical relation.

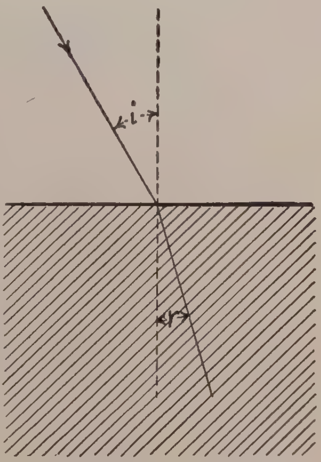

FIG. 30.—Illustrating refraction of light.

It will thus be seen that the conception of index of refraction must involve two substances and that its value will depend upon the density of each. As ordinarily used, it is understood that the light emerges from ordinary air into the substance whose index is being measured and the term "index of refraction" therefore signifies the ratio of the sin of the angle of incidence from air, to that of the angle of refraction in the substance under consideration.

Applications.—In a number of instances the measurement of index of refraction furnishes a means for identifying certain materials and in some cases a quantitative estimation is made possible, where the identity of all components in a given mixture is known. As an example of qualitative testing may be mentioned the measurement of this property in oils and fats. This is discussed in Chap. X. The quantitative determination of alcohols and sugars in aqueous solutions and of milk fat in milk and the detection of added water in milk by the examination of milk serum are familiar examples of quantitative application of refractivity measurements. Some of these are given attention in Chap. XI.

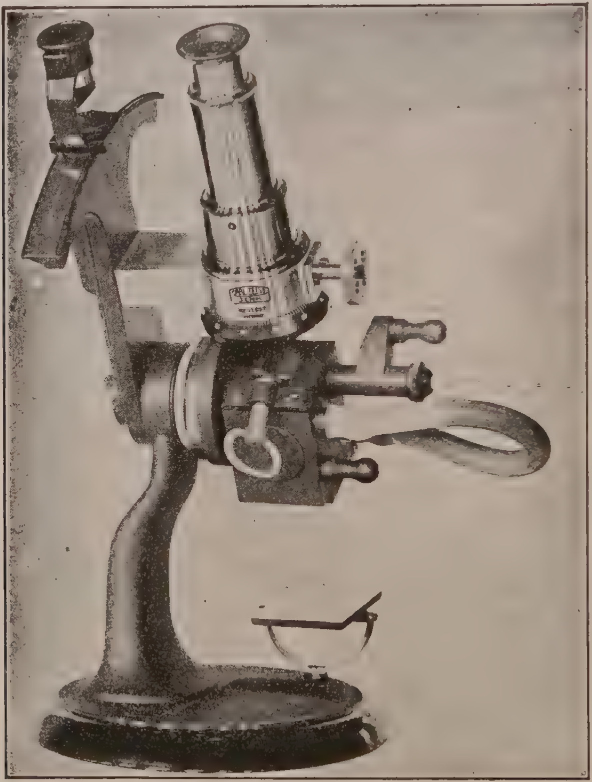

Fig. 31.—The Abbé refractometer.

Light of short waves is refracted more than that of longer waves. Therefore in expressing the index of refraction the character of light must be indicated. Ordinarily the refraction for the D_1 sodium line of the spectrum is understood unless some other light is specified.

Apparatus.—For the determination of refractivity an instrument must provide (*a*) a prism of known index of refraction, a plane surface of this lying against the liquid or solid under investigation and (*b*) an optical system of lenses for examination of the effect of refraction. All other parts may be regarded as accessories, for adding convenience of manipulation or for increasing the accuracy of observations.

Abbé Refractometer.—This instrument (Fig. 31) serves very well for measuring refractive indices of fairly viscous and non-volatile liquids. The optical system is represented in section in Fig. 32. The lower half of the prism (not shown in the figure) serves merely as a means for holding a layer of liquid in contact with the upper half. A mirror, below, reflects light into the system and this passes through the lower prism and the layer of liquid, emerging from the latter into the upper prism at all possible angles. The ray g, g', g'', grazing the lower surface of the upper prism, represents the limiting angle of incidence (90° to the normal) and the angle of refraction of this ray (or of one whose angle of incidence is infinitesimally less than 90°) forms the bounding line between a region of light and one of darkness. This will be seen as the line G in Fig. 32.

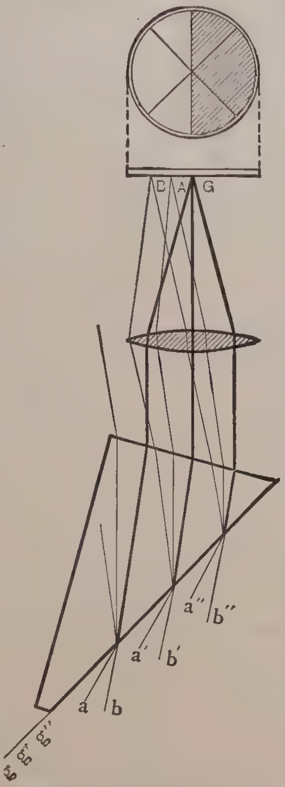

FIG. 32.—Path of rays in the Abbé refractometer.

If the liquid from which light emerges is exchanged for one of different index of refraction the angle of refraction of the grazing ray will be changed. In other words the boundary between light and dark in the field of observation will be shifted. It is the observation of the position of this boundary that constitutes the determination of index of refraction by means of this instrument. The relative positions of telescope and prism system can be altered at will. The prism system is tipped until the bounding line lies upon the cross hairs of the telescope. The index of refraction is then read on an outside scale.

Dispersion.—The refractive effect at the surface separating transparent media varies according to the wave length of the light, shorter waves being most refracted. The result of using polychromatic light in a system like that just discussed will therefore be that the light and dark fields will be separated by a colored zone of spectral tints, instead of by a sharp, uncolored line. It then becomes necessary to use monochromatic light or else to introduce some device for correcting the dispersion. In the Abbé instrument a "compensator" provides this correction.

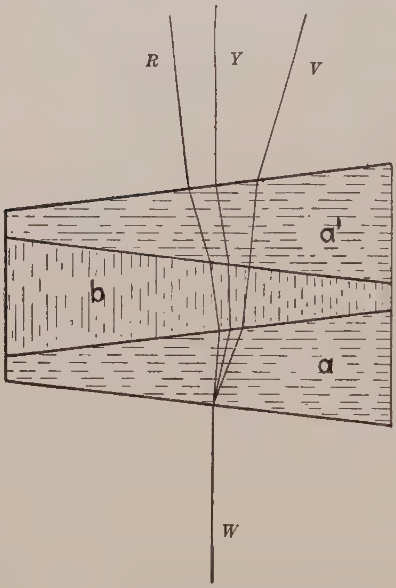

FIG. 33.—Single Amici prism, as used in dispersion compensators.

The Compensator.—The dispersive effect of different transparent media is not a definite function of the index of refraction for monochromatic light. For example, two media might have the same indices of refraction for yellow light but quite different indices for violet light. This principle is utilized in the construction of the Amici prism. In Fig. 33 the parts *a*, *a′* are prisms of crown glass and *b* is of flint glass. The angles and the dispersive powers of these prisms are so related that when they are cemented together the combined effect is to allow the yellow (D_1) ray of entering polychromatic light to pass through with its direction unchanged, while rays of all other wave lengths are refracted.

In other words, the part *b* has a refractive power which is greater for red, smaller for violet, and the same for yellow as the corresponding powers of the combination of *a* and *a′*.

If this Amici prism is placed in the path of light which has been refracted (and dispersed) by the prism-fluid system of the refractometer, it will either add its dispersive effect to that already produced or oppose the latter, according to the way in which it is turned about the axis of the instrument but, no matter what its position, the direction of the yellow ray is unchanged and the reading for index of refraction is not altered, since this value is usually stated in terms of yellow light. If the angle between the planes of dispersion of Amici and refractometer prisms is such that the dispersion of the latter is exactly neutralized, "compensation" is effected and the border line between light and dark fields becomes distinct and without color.

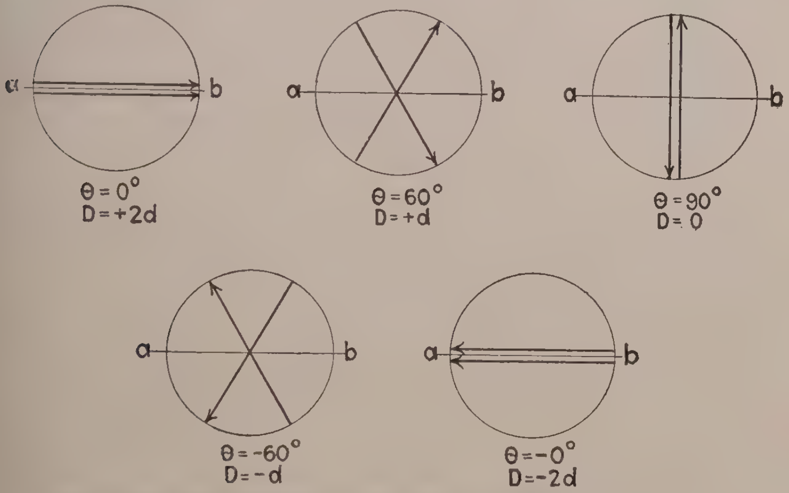

FIG. 34.—Five typical positions of the units of a double compensator, showing net dispersive effect (*D*) in the direction *ab*.

If two Amici prisms are used, as is the case in the Abbé refractometer, and if they are mounted in such a manner as to revolve in opposite directions, the dispersive effect of the combination may be varied between the limiting values of + 2 *d* and − 2 *d*, where *d* is the quantitative effect of one Amici. This widens the range of possible compensation.

The diagrams of Fig. 34 illustrate a few of the possible positions of the two elements of a double compensator, with the net effect of the altered system upon dispersion. In explanation of these diagrams the combined dispersive effect in the direction *ab* is represented by *D* and that of one unit in its own plane (represented by the arrows) by *d*. It is evident that the component of dispersion in *ab* for any given position of a unit varies as the cosin of the angle, θ, between *ab* and the plane of dispersion for the unit.

Any conceivable value between 2 *d* and -2 *d* may be obtained and compensation thus effected for any dispersion within this range.

Butyro-refractometer.—The Zeiss butyro-refractometer has an optical system similar to that of the Abbé instrument. The principal difference is in the fact that the telescope and prism system of the former instrument are rigidly connected so that the divided field cannot be shifted to bring the line of division upon the central crossing of cross hairs. Instead, a scale graduated in arbitrary degrees is fixed within the instrument and the position of the bounding line is read upon this.

As its name indicates, the butyro-refractometer is designed for use in dairy laboratories and its chief function is in the testing of butter. Therefore, instead of being provided with a compensator the prisms of the instrument are "achromatized" for pure butter so that this fat gives no dispersion. This constitutes the basis for an additional qualitative test for butter, since other fats used as substitutes will have different dispersive power and the bounding zone in the field will therefore be spectrally tinted.

Dipping Refractometer.—From an inspection of Fig. 32 it will be seen that the essential parts of the Abbé refractometer, from the optical standpoint, are the upper prism, the objective and an eyepiece for viewing the field. (The latter is not shown in the figure.) The thickness of the liquid film is of no particular moment and the prism might as well be immersed in a quantity of the fluid. In the dipping refractometer (Fig. 35) this principle is utilized. The entire instrument is in one rigidly built piece, the prism being fixed at the lower end. The instrument is hung so that the prism is immersed in a bath of the liquid under examination and the index is read on a scale within.

The compensator for the dipping refractometer consists of a single Amici prism (*C* of Fig. 35) rotated by the milled ring *Y*. The range of compensation is thus less than that of the double compensator but it is sufficient for this refractometer, whose range for indices of refraction is comparatively narrow.

In Fig. 35 two points are arbitrarily selected to represent the entire surface of the prism where light enters. *gg'* is the ray of grazing incidence refracted and focused at *G*, which bears the scale. *a* and *a'* represent rays entering at any other incident angle, focused at *A*. Above is a projection of the field. At the right of *G* the field is dark because the critical ray, refracted as it enters the prism, can make no greater angle of refraction for the given prism-fluid combination. When the fluid is changed for another having a different index of refraction that angle of refraction for the critical ray is changed and the border line within the field is shifted.

Pulfrich Refractometer.—This instrument is especially adapted to use with volatile liquids, although it is suitable also for exact determinations of index of refraction of any liquid whatever. The optical principle is exactly the same as that of the Abbé instrument, light entering the fluid-prism system at grazing incidence and the critical angle of total reflection being measured. The liquid is placed in a glass cup which is cemented to the top of the refracting prism, and which may be covered to prevent evaporation of the liquid. Monochromatic light is used and an observation telescope is swung upon an arm in such a way as to bring the division between light and dark fields upon its cross hairs. A circular scale provides the reading.

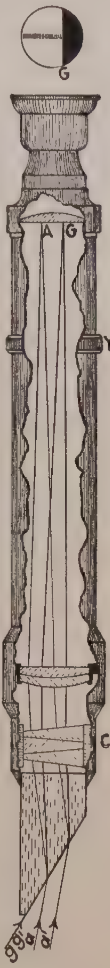

Fig. 35.—Dipping refractometer, partly in section.

On account of the more expensive construction of the Pulfrich refractometer, it is not a common part of the equipment of the technical laboratory. Its more important use is for measurements of refractivity in work of the laboratory of physical chemistry.

Determination of Index of Refraction.—Determine the index of refraction of solutions of sugars or alcohols, furnished by the instructor, and report the per cent concentration, found by reference to tables in the A. O. A. C. "Methods of Analysis" or in other special books, circulars or handbooks. Or use the dipping refractometer for the determination of added water in adulterated milk. For the latter, see page 202, Part III.

CHAPTER VII

OPTICAL ROTATION (POLARIMETRY)

Theory.—In any ordinary beam of light the wave motion is regarded as being transverse and in all possible planes which can include the axis of propagation. When such a beam passes through, or is reflected from, certain transparent media these vibrations are suppressed in all but one plane. The beam of light is then said to be "plane-polarized."

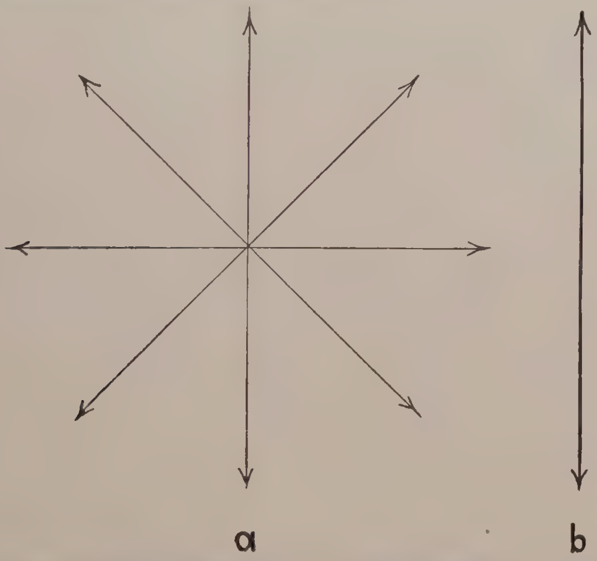

Fig. 36.—Diagrammatic representation of typical planes of vibration of (*a*) unpolarized and (*b*) plane-polarized light.

This change is illustrated in Fig. 36, in which *a* represents some of the planes of vibration of unpolarized light and *b* that of plane-polarized light. In these diagrams the axis of propagation of the beam of light is understood to be perpendicular to the plane of the paper.

Most transparent media permit plane-polarized light to pass through them unchanged but there are certain crystals and solutions that possess the remarkable property of rotating the plane of polarization to the right or left. Further, this is a *quantitative* property and the magnitude and direction of the angle of rotation is a specific property of the substance itself, a given solvent being understood in the case of solutions. This property undergoes a definite change of value with definite changes in temperature and in the case of solutions it varies directly with the concentration. This last is a very important consideration and it will be readily seen that if an instrument can be constructed for measuring the angle of rotation, this will serve as a means for the quantitative determination of optically active substances in solution.

Substances that rotate the plane of polarization to the right are *dextro-rotatory* while those that rotate to the left are *laevo-rotatory*. The angle of rotation varies according to the wave length of the light that is used and it therefore becomes necessary to use monochromatic light in order to have a definite, measurable rotation. Sodium light is generally used for this purpose.

Specific Rotatory Power.—*The angle of rotation which would be produced by a column of solution* 1 *dm long and containing* 1 *gm of the active substance in each cubic centimeter* is known as the "specific rotatory power" or "specific rotation" of a given active substance. This is, in a sense, a hypothetical figure as few solutions could be made of so great a concentration; however, the specific rotation can be calculated from the results of measurements on more dilute solutions. The specific rotation, at 20° and for the *D*-line of sodium light, is expressed by the symbol $[\alpha]_D^{20°}$.

For a solution of an active substance in an inactive solvent, such as water, the angle, α, of rotation is approximately in proportion to the concentration, which may be expressed as grams of active substance in 100 cc of solution. This relation does not hold strictly, on account of changes in electrolytic dissociation (ionization), molecular association (polymerization), hydrolysis, or hydration, with changes in concentration, where any of these factors apply to a given solution. In many of the ordinary applications of the polarimeter to analytical problems the influence of these factors is negligible.

From these considerations the formula:

$$[\alpha]_D^{20^\circ} = \frac{100\ \alpha}{c\ l} \tag{1}$$

is derived, as representing the specific rotation of an active substance in solution, where α is the angle of rotation produced by a column l dm in length and of concentration c gm in 100 cc.

A few examples, out of a very large number of optically active substances, with their specific rotatory powers are given in the following table, in which the sign (+) indicates dextro-rotation and (−) laevo-rotation. The solvent is understood to be water, unless otherwise stated.

Table IV.—Specific Rotatory Powers

Substance	$[\alpha]_D^{20^\circ}$*
Dextrose (grape sugar)	+53.1
Levulose	−93.3
Sucrose (cane sugar)	+66.5
Invert sugar	−20.57
Lactose (milk sugar)	+52.53
Maltose (malt sugar)	+137.5
Starch	+190.0
Tartaric acid (ordinary)	+12.0
Nicotine (in benzene)	−164.0
Cocaine (in chloroform)	−16.3
Quinine sulphate (in alcohol)	−225.7
Camphor (in benzene)	+41.4
Lemon oil (no solvent)	+59 to +67
Orange oil (no solvent)	+96 to +98

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* These figures represent mean values for ordinary concentrations. There is nearly always a certain variation with concentration and where this is large it must be taken into consideration. For example, the specific rotation of levulose is $-88.13 - 0.2583\ c$, and of dextrose $+52.50 + 0.0188\ c + 0.000517\ c^2$, c indicating grams of active material per 100 cc. It must also be noted that a number of substances exhibit "mutarotation," that is, the rotation of the freshly prepared solution changes after a certain lapse of time into the final, stable value.

The Polarimeter.—Listed in what (perhaps somewhat arbitrarily) may be regarded as the order of relative importance, and not in the order in which they are fixed in the instrument, the essential parts of an instrument for measuring optical rotation are as follows:

(*a*) An optical part, *P*, (Fig. 37) for polarizing entering monochromatic light in a definite plane. This is the "polarizer."

(*b*) A part, *A*, similar to the polarizer, which can be placed in the path of the light and rotated about the axis of propagation of the beam. This is the "analyzer." With the polarizer fixed in position there will be a corresponding position of the analyzer which will permit the maximum brightness of transmitted light and another which will cause total extinction, the first representing a coincidence of the planes of polarization of polarizer and analyzer, the second an inclination of 90° for these planes.

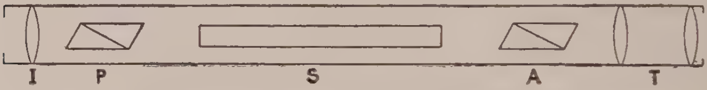

Fig. 37.—Diagrammatic representation of the essential parts of a simple polarimeter.

(*c*) A tube, *S*, to contain the solution under investigation which can be placed in the light path, between the analyzer and the polarizer. This tube must be of definite length and it must have plane, transparent ends, placed perpendicularly to the direction of light travel.

(*d*) A lens, *I*, for directing parallel light into the instrument.

(*e*) A system of lenses, *T*, through which the operator may observe the action of the first four parts.

(*f*) A circular scale upon which is indicated the angle through which the analyzer is rotated.

The relative positions of these parts are shown in Fig. 37.

Making a Reading.—Briefly stated, the determination of rotatory power with the most simple instrument possible would be made as follows:

The analyzer is brought into a relation with the polarizer, such as to permit the maximum transmission or extinction of light. This establishes the zero point of the instrument. The tube of solution is then placed in position and the analyzer is turned so that its plane of polarization lies in the new plane, which has been rotated by the solution from its original position. The magnitude and direction of the angle, α, through which the analyzer has been turned, is noted and from this and from the length of column and the known concentration of the solution the specific rotation is calculated.

In the more common case the specific rotation of the substance is already known and the concentration of the active substance in the solution is the factor in question. For example, the per cent of cane sugar in a syrup is to be determined. A definite weight of the syrup is diluted to a definite volume and the angle of rotation produced by a column l dm in length is determined. The specific rotation of sucrose is given as +66.5. We have then the equation (from Eq. (1), page 123):

$$66.5 = \frac{100\,\alpha}{cl}, \text{ or } c = \frac{100\alpha}{66.5\,l}.$$

The length, l, is known and the angle, α, is determined by observation. The concentration, c, of sugar in the solution, as well as the concentration of sugar in the original syrup, is then easily calculated.

Construction of Polarizer and Analyzer.—In the most common form of this instrument the polarizer and analyzer are of identical construction. It is a well known fact that when a ray of light falls perpendicularly upon certain faces of a crystal of Iceland spar (natural, crystallized calcium carbonate) the light is broken into two rays which are unequally refracted, so that when any object is viewed through such a crystal two images are observed. What is equally important is that these two rays are polarized in planes perpendicular to each other.

The Nicol Prism.—This is made by cutting a crystal of Iceland spar into two wedge-shaped pieces and grinding the faces in such a manner that when these pieces are cemented together one of the plane-polarized rays may pass through while the other will be reflected to the side of the prism and there absorbed by a blackened surface. In Fig. 38 the incident ray, W, is double-refracted and at the dividing surface between the two parts of the crystal, the "ordinary" ray, o, is reflected to the side of the prism while the "extraordinary" ray, *e*, passes through. This ray is polarized in a plane which is perpendicular to the "optical principal plane" of the prism, a term which need not be defined here.

This Nicol prism, properly fixed in place in the end of the polarimeter nearest the light source, forms the polarizer. The analyzer is another Nicol of similar construction. When this is turned so that the optical principal plane is parallel to that of the polarizer, maximum brilliancy of transmitted light is observed. If these two planes are perpendicular to each other, total extinction results because the *extraordinary* ray from the polarizer is now in the plane for the *ordinary* ray for the analyzer and it is therefore reflected to the side of the latter and there absorbed.

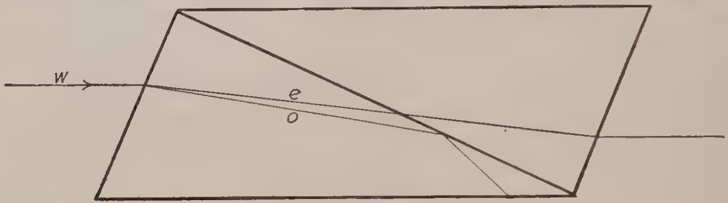

FIG. 38.—Nicol prism.

Method of Making Observations.—In practice it is not easy to determine when either maximum brightness or maximum extinction of entering light occurs. Accordingly most polarimeters are constructed with an additional device to aid in making the reading. In "half-shadow" instruments the field is divided into halves by interposition of a thin plate of quartz which covers half of the diaphragm of the polarizer. The thickness, method of grinding and position of this plate are such as to cause a small difference between the angles of maximum intensity or extinction for the two halves. That is, as the analyzer is rotated, one half of the field gains in intensity while the other half diminishes. The zero of the instrument is the position of the analyzer which gives a uniformly lighted field.

By use of a somewhat similar principle triple fields may be produced. The arrangement of the Nicol prisms is different in instruments using this principle but the effect is such that the field is divided into three parts. The sides have always like intensities and these brighten as the middle section darkens. Here again, the field of uniform intensity is seen at the zero angle.

Light Source.—Many of the forms of polarization apparatus are constructed for monochromatic light of a specified wave length. White light cannot be used with such an instrument because its component rays suffer different rotation of their polarization planes, according to their wave lengths, the shorter waves being rotated to the greatest degree. Sodium light is most commonly used for this purpose as it contains rays from a very narrow band in the spectrum and it is therefore nearly homogeneous. A sodium light is easily produced by placing any suitable sodium compound in a non-luminous flame. Sodium carbonate or recently fused sodium chloride is suitable for this purpose. The salt is placed in a platinum spoon or fused into a bead in a loop of platinum wire, or some similar device may be employed.

Quartz Wedge Compensation: The Saccharimeter.—It has been stated in the preceding paragraph that the rotation of the planes of polarization varies for light of different wave lengths. If white light is used to illuminate the ordinary polarimeter the effect of interposing an active substance in the path of the rays is a dispersion of the various polarization planes. This is analogous, in a manner, to the dispersion of white light by refraction and it was seen in the discussion of the refractometer (page 116) that this dispersion could be corrected by optical means without altering the refraction of a given ray, such as the yellow one.

The quartz wedge compensator for the polarimeter makes possible the use of white (polychromatic) light. Quartz is optically active and it occurs in both dextro- and laevo-rotatory forms, the angle of rotation of sodium light at 20° for a plate 1 mm thick being ±21.72°. If absolutely similar plates of right and left rotating quartz should be placed between polarizer and analyzer, the net effect would be zero rotation. If the thickness of either one of these could be varied at will the effect of the combination could be made either right or left rotating, within certain limits, and this effect might be made such as to compensate (neutralize) exactly the rotation of a solution which is placed in the instrument and whose rotation is to be measured. In such a case both polarizer and analyzer might be made as rigid, stationary parts of the instrument, the only adjustable part being one of the quartz plates. This possibility of adjustment is accomplished by cutting one of the plates diagonally, making two wedge-shaped pieces which may be thrust past one another by means of an appropriate screw, the magnitude of the effect being noted upon a scale.

Now it happens that the rotation *dispersion* of quartz for white, or other polychromatic, light is nearly identical with that of cane sugar in solution. Since, in using this instrument, the quartz wedge combination will always be adjusted to be equal in rotatory power to that of the solution being investigated, *but in the opposite direction*, it will also be true that the *dispersion* of the sugar solution will be nearly compensated by the opposite, but otherwise nearly equal, dispersion of the quartz system. Because of these relations the instrument constructed in this manner is known as a "saccharimeter." If used with other solutions than those of cane sugar the polarization dispersion could be compensated only approximately, at best, and readings of the angle of rotation could not be correct. In such a case it would be necessary to use sodium light or a selective light filter.

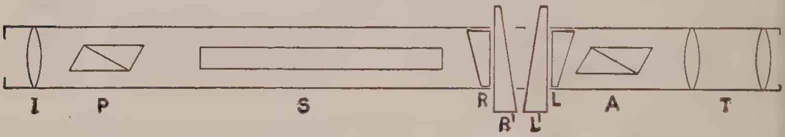

FIG. 39.—Diagrammatic representation of the essential parts of a quartz wedge saccharimeter, having double compensating wedges.

The relations of the optical parts of the quartz wedge saccharimeter are shown diagrammatically in Fig. 39.

Light Filter for Use with the Saccharimeter.—The quartz wedge system fails to give *exact* compensation for the rotation dispersion of sugar solutions and in order to avoid slightly high readings it is necessary to absorb a part of the blue and violet waves from white light, as these suffer the greatest dispersion. The International Commission for Unifying Methods of Sugar Analysis adopted the suggestion of Bryan[1] that white light shall be passed through a solution of potassium dichromate "of such concentration that the percentage content of the solution multiplied by the length of the column of solution in centimeters is equal to nine."

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[1] *J. Ind. Eng. Chem.*, **5**, 167 (1913).

The Sugar Scale.—The simplest and most generally useful scale for the polarimeter is the circular scale, divided into angular degrees, with a vernier for greater accuracy in reading. But in the practical use of the instrument for analytical purposes there arises (as is usually the case when scientific instruments are used for practical testing) a demand for a direct-reading scale that can be interpreted in terms of the per cent of active substance, without calculations other than of the simplest sort. The largest commercial use of the polarimeter is for sugar testing and for this purpose there have come into general use three scale systems: the Ventzke (German), the Laurent (French) and the International, the latter being a development of the Ventzke scale. A scale of one of these types is usually placed upon the instrument, even when angular degrees also are indicated.

The Ventzke Scale and the Normal Weight.—In this system a "normal" solution of cane sugar was first defined as one having a specific gravity of 1.100 at $\frac{17.5°}{17.5°}$. Of course this is an entirely arbitrary value but it served to fix the basis for the system. The scale values were fixed by polarizing a solution of this concentration in a 200-mm tube at 17.5°C., this defining the 100° point on the scale. Because of the difficulties involved in preparing solutions having this exact concentration by use of the hydrometer alone, it became customary to make the normal solution for fixing the scale points by weighing 26.048 gm of sucrose and making the solution to 100 cc at 17.5°. This is the same as Ventzke's solution. The "normal weight" was then 26.048 gm.

The adoption of the Mohr unit of volume (1 cc "Mohr" = 1.00234 true cc) brought confusion into the scheme, as instrument builders for a time used the old normal weight with the new volume unit. The 100° point on the Ventzke scale was then fixed[1] "by polarizing in a 200-mm tube a solution containing 26.048 gm of sucrose, weighed in air with brass weights, in 100

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[1] U. S. Bureau of Standards, Circ. **44,** 27, 2nd ed. (1918).

Mohr cc at 17.5°, the temperature of the quartz wedges, as well as the polarizing temperature, being 17.5°. This confusion has been still further increased by the more recent readoption of the true cubic centimeter as a unit for practically all scientific work (volumetric apparatus now being furnished by the manufacturers, graduated upon this basis) and by the fact that there is frequently no indication upon the instrument as to what unit has been used in working out the scale. And it may be well to remark here that the all too general custom in industrial (and some college) laboratories of using all commercial volumetric and other apparatus, and even weights, without calibration leaves the accuracy of much analytical work in a very questionable light. The only way by which accuracy can be assured is by calibrating the flasks, burettes, pipettes and weights to be used in this work and by checking the saccharimeter scale against quartz plates that have been tested by the Bureau of Standards or by other competent standardizing bureaus.

The International Scale.—In 1900 the International Sugar Commission recommended that the sugar scale be redefined, basing the 100° point upon the true cubic centimeter and a temperature of 20° C. Introducing the correction for the change of volume unit, and of the specific rotation of sucrose, the expansion of the glass polarizing tube, quartz wedges and metal scale, between 17.5° and 20°, the normal weight of sucrose becomes 26.000 gm. The International sugar scale is then to be defined as follows: "*The graduation of the saccharimeter shall be made at 20° C., 26 gm of sucrose dissolved in water and the volume made up to 100 metric cc. All weighings are to be made in air with brass weights, the completion of the volume and the polarization are to be made at 20° C. This will determine the 100° point.*"

In order to determine the per cent of sucrose in a material of unknown purity is only necessary to weigh 26.000 gm of the sample, dissolve and dilute to 100 cc and then "polarize" in a 200-mm tube. If the material were pure cane sugar the reading would be 100° International (100° I.). If it were 50 per cent pure the reading would be 50° I. In general, then, degrees on this scale indicate per cent of sucrose. Of course it is essential that no other active substance shall be present in the solution or that some method for accounting for these shall be available.

In order to make a simple reading possible it is not necessary to use the normal weight of sample or to polarize in a 200-mm tube. Any simple fraction or multiple of these numbers may be employed and due account taken in the calculation. Polarization tubes are provided, varying by even stages from 100 to 400 mm in length.

The sugar scale provides direct readings for other sugars and for other optically active substances, not sugars, by use of a properly modified normal weight. Thus for lactose (milk sugar) $[\alpha]_D^{20°} = 52.53$, instead of 66.5 as for sucrose. Therefore it will require $\frac{66.5}{52.53} \times 26 = 32.9$ gm of lactose in each 100 cc to give a rotation of 100° on the sugar scale. 32.9 gm is then the normal weight for lactose and if, for example, 32.9 gm of milk were treated in such a manner as to obtain the clear serum and this diluted to 100 cc and then polarized in a 200-mm tube, degrees International would indicate directly the per cent of lactose in the milk. This determination is described in the section on Dairy Products, page 214, Part III.

The Laurent Scale.—This is constructed so that a quartz plate 1 mm in thickness and cut so that its faces are perpendicular to the optical axis will give a rotation of 100° L. The normal weight for this scale will then be such that when this quantity of substance is dissolved in 100 cc and the solution is polarized in a 200-mm tube it will give the same rotation as a quartz plate of the above description. Because of small differences in the specific rotation of quartz specimens there has been some uncertainty regarding the normal weight. The value now accepted for sucrose is 16.29 gm, dilution to 100 true cc being understood. Both Ventzke and Laurent scales are falling into disuse, being properly replaced by the more rational International scale.

The Common Sugars.—Sucrose, or cane sugar, is the principal sugar of the juices of sugar cane, sorghum, beets and many fruits. It may be converted into a mixture of equal parts of dextrose and levulose by hydrolysis, induced by action of acids:

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Levulose}}{C_6H_{12}O_6}.$$

The difference between the molecules of dextrose and levulose is a structural one and this has a direct bearing upon the rotation of these sugars. The values for the specific rotation (for 20-per cent solutions) of sucrose, dextrose and levulose are +66.5, +53.1 and −93.3, respectively. The mixture of dextrose and levulose has a specific rotation, for these concentrations, of −20.57, which is practically the mean of the separate values for the two sugars. Because of the change in the direction of rotation with this conversion of sucrose, the reaction is known as one of "inversion" and the resulting mixture of sugars is called "invert sugar."

Cane Sugar.—Sucrose can be determined by a single polarization only in case no other active substance is present in the solution. In case either dextrose or invert sugar is present a polarization before and after the inversion of cane sugar gives the necessary data for the calculation of sucrose by the modified Clerget formula. This formula is derived from the following considerations:

From the values for $[\alpha]_D^{20°}$, given above:

$\frac{66.5}{20.6} \times 26 = 83.9$. Therefore 83.9 gm is the normal weight (International) for invert sugar.

From the equation for inversion: 26 gm of sucrose yields 27.37 gm of invert sugar and this is $\frac{27.37}{83.9} = 0.3262$ of the normal weight. (Herzfeld's value, 0.3266 is now generally used.)

If the normal weight of sample (based upon sucrose) has been used for making the solution for the direct polarization (P), then each per cent of sucrose in the sample will give a rotation of +1° (International scale) before the inversion and −0.3266° after inversion. Therefore the *change* of rotation ($P - I$) would be 1.3266° for each per cent of sucrose. If S = per cent of sucrose,

$$S = \frac{P - I}{1.3266}. \qquad (1)$$

This is for a temperature of 20° and it is found that between 0° and 20° the left rotation of invert sugar produced from the sucrose of a normal solution decreases 0.005° for each per cent, for 1° C. rise in temperature. This is chiefly due to a decrease in the rotatory power of levulose. At 0° C. the formula would then read:

$$S = \frac{P - I}{1.4266} \qquad (2)$$

and, in general

$$S = \frac{P - I}{1.4266 - 0.005\,t} \tag{3}$$

This is usually written:

$$S = \frac{100(P - I)}{142.66 - 0.5\,t} \tag{4}$$

t indicating temperature in degrees Centigrade.

Recent work[1] at the Bureau of Standards has shown that the Clerget divisor should be 143.25 instead of 142.66, in presence of the acid used to cause inversion.

The method is applicable only to materials containing no other compounds whose activity is changed by treatment with acids. Molasses from beets and, to some extent, beet sugar contain certain quantities of raffinose ($C_{18}H_{32}O_{16}$), a sugar whose specific rotation is +104.5°. This rotation is diminished by one-half by warming with dilute acids. (See page 136.)

Commercial syrups of various kinds usually possess a color which interferes with transmission of light and makes polariscopic readings difficult. This color is due to a variety of colored organic substances and to caramel formed during the heating processes. It can be removed in most cases by addition of a basic lead salt, of which basic lead acetate is most suitable, or of "alumina cream," a suspension of colloidal aluminium hydroxide in water, freshly prepared. In the case of lead salts the action is partly chemical and partly physical. Complex lead salts of organic acids are formed and these, being colloidal in character, flocculate and carry with them other colloidal colored compounds. Neutral lead acetate is used in some cases where a basic reaction is to be avoided.

Correction for Volume of Precipitate.—In the method as usually followed the clarified solution is diluted to 100 cc before filtration. This ignores the volume of the precipitate and an error is introduced from this source. However, the actual volume occupied by this precipitate is much less than the apparent volume, owing to its colloidal nature. If there is produced a larger quantity of precipitate than can safely be ignored the double dilution method of correction is used. In this method one polarization is made on the clarified solution which has been

[1] U. S. Bur. Stand. Sci. Paper, **375** (1920).

diluted to 100 cc, as usual. Another sample of the normal weight is clarified and diluted to 200 cc and the filtrate is polarized.

Let P = true polarization of a normal solution,
P_1 = polarization of the solution in the 100-cc flask,
P_2 = that of the solution in the 200-cc flask and
v = actual volume of solid precipitate.

Then

$$P_1 = \frac{100\ P}{100 - v}, \tag{1}$$

$$P_2 = \frac{1}{2}\left(\frac{200\ P}{200 - v}\right) = \frac{100\ P}{200 - v}, \tag{2}$$

$$P_1P_2 = \frac{(100\ P)^2}{(100 - v)(200 - v)}, \tag{3}$$

$$P_1 - P_2 = \frac{100\ P(200 - v) - 100\ P(100 - v)}{(100 - v)(200 - v)} = \frac{(100)^2 P}{(100 - v)(200 - v)}. \tag{4}$$

From Eqs. 3 and 4,

$$\frac{P_1P_2}{P_1 - P_2} = P. \tag{5}$$

Therefore the true rotation is the product of the two readings, divided by their difference.

The Association of Official Agricultural Chemists has placed the arbitrary limit of 1 cc of precipitate from 26 gm of sample, for the solution for which no correction need be made.

Determination of Sucrose in a Commercial Syrup.—Prepare a solution of basic lead acetate by one of the following methods:

(*a*) Add 215 gm of neutral lead acetate and 65 gm of litharge (PbO) to 500 cc of distilled water (or a smaller amount using the same proportions) and boil in a covered dish for 30 minutes. Cool, decant the clear solution and determine the specific gravity by means of a hydrometer. Dilute until the specific gravity is 1.25, using recently boiled water.

(*b*) Make the solution directly from dry basic lead acetate and dilute until the specific gravity is 1.25.

Direct Polarization.—Weigh a small dish, then drop in and weigh 26.000 gm of the commercial syrup or molasses. Rinse into a 100-cc volumetric flask with about 50 cc of water and then carefully add basic lead acetate solution until the sugar solution is decolorized as far as any effect can be noticed, avoiding an unnecessary excess of the clarifying agent. Dilute to the mark on the flask, mix thoroughly and filter through a dry filter, rejecting the first 15 cc. Polarize at a temperature of exactly 20°, using a 200-mm tube unless the solution is still colored enough to make this difficult, otherwise use a 100-mm tube and double the reading. This is the quantity P in the Clerget formula (4) on page 133.

Invert Polarization.—Precipitate the lead from the clarified sugar solution by adding either powdered anhydrous sodium carbonate or powdered anhydrous sodium oxalate, very carefully, until a very slight excess is indicated by failure to produce more precipitate. Filter on a dry filter to remove the lead salt. Reject the first 15 cc and save the remainder of the filtrate.

Pipette 50 cc of the clear, lead-free filtrate into a 100-cc flask of ordinary form. If sodium carbonate has been used for precipitating lead, carefully neutralize the excess with hydrochloric acid. Invert the sucrose by one of the following methods:

(*a*) Add 25 cc of water and then add from a pipette 5 cc of concentrated hydrochloric acid, dropping the acid slowly and mixing by rotating the flask. Place the flask in a water bath which is kept at 70°. The temperature of the solution should reach 69° in about 3 minutes. After the flask has been in the bath for 10 minutes remove and cool to 20° in running water. Rinse into a 100-cc volumetric flask, dilute to the mark and mix.

(*b*) Add 5 cc of concentrated hydrochloric acid, slowly and mixing well. Set the flask aside for 24 hours at a temperature of 20 to 25°, or for 10 hours at somewhat above 25°. Dilute to 100 cc and mix.

On account of the considerable variation of the specific rotation of levulose with temperature it is necessary to polarize at a constant, definite temperature. For this purpose a water jacketed tube is used and water at 20° is circulated.

Since the dilution of the solution was doubled after the direct polarization, the reading for the invert polarization is multiplied by 2 if a 200-mm tube is used, or by 4 for a 100-mm tube. Calculate the per cent of sucrose in the syrup.

For low concentrations of invert sugar the variation of rotation with concentration is such that formula (4) on page 133 will not apply. The official method specifies the following formula, where the concentration of sugar in the invert solution is less than 12 gm per 100 cc:

$$S = \frac{100(P - I)}{142.66 - \frac{t}{2} - 0.0065\left[142.66 - \frac{t}{2} - (P - I)\right]}. \qquad (5)$$

This might be further simplified to

$$S = \frac{100(P - I)}{141.73 - 0.4967\,t + 0.0065(P - I)}. \qquad (6)$$

In these formulas the symbols have the significance expressed in the Clerget formula.

Beet Products.—The interference of raffinose in the calculation of sucrose from direct and invert polarizations has already been noted. If the direct polarization reads more than 1° I. higher than the sucrose calculated as already described, the presence of raffinose is indicated. In this case sucrose and raffinose are calculated by the formula of Herzfeld:

$$S = \frac{0.5124\,P - I}{0.839}, \tag{7}$$

$$R = \frac{P - S}{1.852}, \tag{8}$$

R indicating the per cent of raffinose and the other symbols having their former significance.

Commercial Glucose.—This substance contains dextrin and maltose in variable quantities, in addition to the essential dextrose. The specific rotation of the various dextrines is usually about +193 and that of maltose is about +138, that of dextrose being only +53. On this account the specific rotation of commercial glucose is somewhat variable but it is always higher than that of dextrose. The investigations of Leach[1] indicate that +175° I. is the average rotation for a solution containing 26 gm of commercial glucose in 100 cc polarized in a 200-mm tube. From this is deduced the formula:

$$G = \frac{100(a - S)}{175}, \tag{9}$$

a indicating the direct polarization in International degrees, S the per cent of sucrose, determined as already directed, and G the per cent of commercial glucose polarizing +175° I. If invert sugar is present the formula is inapplicable. In this case use is made of the fact that the left rotation of invert sugar decreases with rising temperature, becoming zero at 87° C. At this temperature the mean rotation of commercial glucose has dropped to +163° I., so that the calculation is made by the formula:

$$G = \frac{100\,I}{163}, \tag{10}$$

where I is the corrected invert reading at 87° C.

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[1] *U. S. Dept. of Agr. Chem. Bull.* **65**, 48.

Detection of Invert Sugar.[1]—Dissolve about 20 gm of sample and dilute to 100 cc. Clarify, if necessary, before diluting. Filter and add a slight excess of sodium carbonate. Filter again if not clear. To 50 cc of the solution in a casserole add two drops of a 1-per cent solution of methyl blue and boil. If the color disappears after 1 minute, at least 0.01 per cent of invert sugar is present. If not completely decolorized after boiling for 3 minutes no invert sugar is present.

Determination of Commercial Glucose in Syrups Containing Invert Sugar.—Prepare and clarify, if necessary, the solution of molasses or syrup, following the directions already given on page 134. Invert and obtain the invert reading at a temperature of 87°, using the water jacketed tube for this purpose. Calculate the per cent of commercial glucose by dividing the invert reading (corrected for dilutions) by 163 and multiplying by 100, as indicated in formula (10), page 136.

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[1] Leach, "Food Inspection and Analysis," 4th Ed., 613.

CHAPTER VIII

HYDROGEN ION CONCENTRATION

In the chapter on indicators, in Part I, it was noted that the color change of the indicator bears a definite relation to the changing hydrogen ion concentration in the solution, and that upon this consideration rests the suitability of an indicator for a given titration. The investigation of hydrogen ion concentration must necessarily precede this quantitative knowledge of color changes and such investigations may be made with a high degree of accuracy.

Methods.—A number of methods have been used for the determination of hydrogen ion concentration. Of these, two will be mentioned. These are the potentiometer method and the indicator method. The manipulative details, necessary precautions and the sources of error of these determinations lie outside the scope of this book. The brief discussion here interposed is provided in order to give the student an idea of the general principles involved in the laboratory methods and of the importance of such measurements to the problems of the agricultural chemist. For a full discussion and detailed directions for the determinations, refer to the numerous papers in the journals and to Clark's book, "The Determination of Hydrogen Ions." In this book is a tolerably complete bibliography of the various papers that have appeared on the subject.

The Potentiometer Method.—In principle, this method depends upon measuring the electromotive force of a system in which are placed (*a*) a hydrogen electrode immersed in a solution of known hydrogen ion concentration, (*b*) a hydrogen electrode surrounded by the solution whose P_H value is to be measured and (*c*) a potentiometer. From the measured e.m.f. of the system and the known hydrogen ion concentration around the standard electrode, the ion concentration in the unknown solution is calculated, using the equation:

$$\text{e.m.f.} = 0.059 \log \frac{C}{C'},$$

where C and C' represent the hydrogen ion concentrations in the two solutions.

In practice, corrections must be applied to the above formula in order to account for certain effects not here discussed. It is possible also to substitute for the standard hydrogen electrode certain other well known forms of standard electrodes, such as the "calomel" electrode, in which case the measured e.m.f. has to be corrected for the difference between the potentials of the calomel and the hydrogen electrodes.

The Indicator Method.—This method may be used with greater convenience and at less expense for equipment than the potentiometer method, although it should be recognized that the latter is the fundamental method and that the indicators must themselves have been standardized, usually by the potentiometer method. For the test, there must be provided a series of indicators of which the color corresponding to a given hydrogen ion concentration is known, and extending over a wide range of P_H values. A set of "buffer" solutions is prepared, these being solutions of certain salts or acids whose hydrogen ion concentration is definite and known and which are easily reproduced. By matching the color produced when definite quantities of suitable indicators are added to the solution under investigation, with those produced by the same indicators and the various buffer solutions, the P_H value of the former is determined.

The indicators listed below are the selection of Clark and Lubs and their preparation and use are described in detail by Clark, in his work above cited.

TABLE V.—INDICATORS

Common name of indicator	Color change, acid to base	P_H range
Thymol blue (acid range)	Red-yellow	1.2 – 2.8
Brom phenol blue	Yellow-blue	3.0 – 4.6
Methyl red	Red-yellow	4.4 – 6.0
Brom cresol purple	Yellow-purple	5.2 – 6.8
Brom thymol blue	Yellow-blue	6.0 – 7.6
Phenol red	Yellow-red	6.8 – 8.4
Cresol red	Yellow-red	7.2 – 8.8
Thymol blue (basic range)	Yellow-blue	8.0 – 9.6
Cresol phthalein	Colorless-red	8.2 – 9.6

The series of buffer solutions suggested by Clark and Lubs consists of mixtures of hydrochloric acid with potassium chloride and with potassium acid phthalate, and of sodium hydroxide with potassium acid phthalate, with monopotassium orthophosphate and with orthoboric acid. By mixing these in stated proportions and at stated dilutions the P_H range is covered from values of 1.2 to 10.0, in steps of 0.2.

Gillespie has described[1] a method for dispensing with the use of buffer solutions.

Applications.—A high degree of importance is attached to the application of P_H values to problems of agricultural and biological chemistry. Mention may be made of the bearing of soil acidity upon productiveness and upon adaptation to different crops; of acidity of plant juices upon plant health and disease; and of acidity of milk upon butter and cheese production. Hydrogen ion concentration is of importance also in the culture and study of bacteria, yeasts and molds; in the study of physiological chemistry, particularly with relation to the digestive system and the blood. Many other applications might be noted, of not so direct interest to the agricultural or biological chemist and many interesting lines of research have been opened up by the high degree of development of this line of testing.

[1] *Soil Science*, **9**, 115 (1920); *J. Am. Chem. Soc.*, **42**, 742 (1920). See also MEDALIA: *J. Bact.*, **5**, 441 (1920); **7**, 589 (1922), and BARRETT AND BARRETT: *Proc. Soc. Exptl. Biol. Med.*, **18**, 127 (1921).

PART III

ANALYSIS OF AGRICULTURAL MATERIALS

The following chapters constitute an introduction to the application of quantitative analysis to the solution of agricultural problems. The subjects treated are typical phases of the broad field of agricultural analysis. The student is especially cautioned that if he is to avoid the common danger of falling into ways of mere mechanical routine he must, here as elsewhere, cultivate the habit of looking for the scientific principles underlying his work, as well as for the significance of its results in connection with scientific agriculture.

CHAPTER IX

FEEDS

The raw materials of feeds vary greatly in their composition, their feeding and commercial values depending upon their content of protein, fat, mineral matter, carbohydrates and vitamins and upon the ease with which food elements are digested and assimilated. There is much difference in the feeding value of protein and fat, according to the sources from which they are derived. The degree of utilization of these products can not always be measured with exactness by chemical means but it must be determined from feeding trials with animals. However, chemical analysis furnishes the best available means for estimating approximate feeding values from percentage composition. This is especially true of commercial ready-mixed feeds, which are often made up from many different plant and animal sources; chemical analysis furnishes the only quick method for determining their approximate commercial value.

Composition of Some Common Feeds.—If the feed is made from the whole grain the composition of the groups will be about as represented in the table below and if made up of grain by-products, with the more valuable parts taken out and substituted with cheaper materials, it is often possible to detect the deception by analysis.

The analysis of feeds commonly includes the determination of moisture, ash, crude fat, crude fiber, crude proteins, carbohydrates and pentosans. The entire carbohydrate group is often expressed as "nitrogen-free extract," which is obtained by deducting the sum of all other groups from 100.

Most states now have laws which control the manufacture and sale of feeds. These laws usually require a guarantee of the per cent of *ether extract*, *crude protein*, *fiber* and *ash*. The average composition of the principal cereal grains is tabulated as follows by Villier and Collin.

Table VI.—Average Composition of Principal Cereals

	Wheat	Barley	Rye	Oats	Rice	Corn	Millet	Buckwheat
Water	13.65	13.77	15.06	12.37	13.11	13.12	11.66	12.93
Crude protein (Nitrogenous substances)	12.35	11.14	11.52	10.41	7.85	9.85	9.25	10.30
Crude fat	1.75	2.16	1.79	5.32	0.88	4.62	3.50	2.81
Sugar	1.45	1.56	0.95	1.91	.....	2.46	.....	....
Gum and dextrin	2.38	1.70	4.86	1.79	16.52	3.38	65.95	55.81
Starch	64.08	61.67	62.00	54.08	.....	62.57	.....	....
Crude fiber	2.53	5.31	2.01	11.19	0.63	2.29	7.29	16.43
Ash	1.81	2.69	1.81	3.02	1.01	1.51	2.35	2.72

Method of Sampling.—Commercial feeds are usually shipped to the consumer in sacks and it is important that the samples chosen from them shall be representative of the feed contained in all parts of the sack. A sampler somewhat similar to that used for fertilizers (Fig. 59, page 273), but larger in diameter than this, may be forced to the bottom of the sack of feed, the slide covering the opening in the tube being moved to the side and tapped so that the tube can fill with feed. The slide is again closed, the tube is withdrawn from the feed sack, and the contents of the sampler placed on a sheet of paper and thoroughly mixed. About a pint of this uniformly mixed feed is saved for analysis.

Preparation of Sample.—The sample should be ground in a feed grinder (Fig. 40, suitable for grinding coarse feed materials) so that it will pass a sieve having openings 1 mm in diameter (0.04 in). Sometimes it is quite difficult to reduce it to this degree by grinding, in which case it should be made as fine as possible by any other available means. A container which may be tightly stoppered should be provided to hold the sample. At the start, enough should be prepared and mixed to serve for the entire analysis. This will require about 200 gm.

Moisture.—There are several minor factors which tend to modify the results of moisture determinations. One of these is the loss of essential oils and other volatile bodies during the drying process. Partly compensating this is a possible gain in weight due to oxidation of fats and sugars, when drying takes place in the air. As these changes are variable the method of drying in air at elevated temperatures has been abandoned. If a temperature of 100° is to be used it is necessary to have available an oven for drying at reduced pressure or in an atmosphere of an indifferent gas, such as hydrogen.

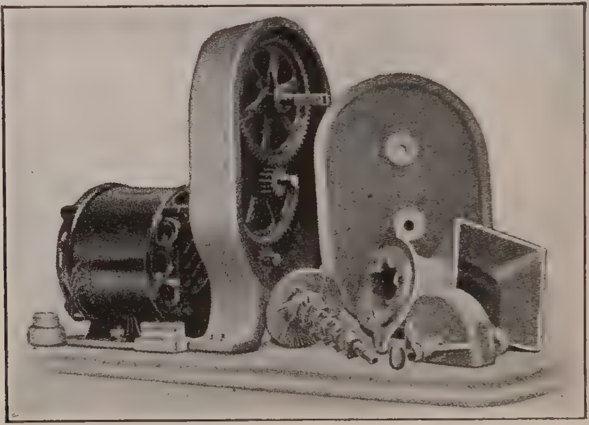

FIG. 40.—One form of grinder for coarse feeds. (Shown disassembled.)

Feeds dried at ordinary temperature under reduced pressure usually show about 1 per cent less moisture than is found by a direct heating method. About four to six days is usually required to obtain constant weight by the reduced pressure method, even if the sulphuric acid used in the desiccator is changed several times. The special advantage of the method lies in the thorough desiccation of the sample without the possibility of chemical changes brought about by heating and oxidation but the length of time required for the experiment makes the method impracticable for all work except that requiring a high degree of refinement and accuracy.

Determination of Moisture: *At* 100°.—Weigh about 2 gm of the feed in a weighed flat dish or, in case the fat is to be extracted, in a weighed alundum cup. Place in an oven which can be exhausted or through which dry hydrogen can be circulated and heat at 100° for at least five hours or until the weight is constant. After the sample has cooled in a desiccator it should be weighed as rapidly as possible in order to avoid undue exposure to moist air. Preserve the dried sample for the crude fat determination (page 147), or for the ash determination, described below.

At Room Temperature.—Place 2-gm samples in separate 6-inch vacuum desiccators (see Fig. 8, page 28) containing 200 cc of fresh concentrated sulphuric acid and exhaust to a pressure of 1 mm by means of a pump. It will require about four to six days drying to secure constant weights. The desiccators should be rotated gently several times a day in order to mix the lower, more concentrated sulphuric acid and the upper layers that have become diluted by absorbed moisture. After 24 hours drying, carefully open the desiccator and weigh the sample. Place in a desiccator containing fresh sulphuric acid and repeat the process of drying and weighing until the weight becomes constant. Calculate the total loss as moisture. Preserve the dried sample for the crude fat determination (page 147), or for the ash determination, described below.

Ash.—The ash determination requires much patience. The high carbon contained in oily seeds is very hard to oxidize so as to secure a white or gray ash but too high a temperature will cause volatilization of certain ash constituents, such as chlorides of the alkali metals. The ash should contain the mineral compounds (such as calcium phosphate, potassium or sodium chloride and some silicon compounds) of the plant tissues and sap. Some phosphorus and sulphur may be present as part of the protein molecule and these may be volatilized but they are not properly to be considered as part of the ash unless they are normally left upon burning (as phosphates or sulphates). This is usually the case in grasses and leaves but not in seeds.

Determination of Ash.—Either the dried sample obtained in the moisture test or a new undried sample may be used for this determination. Ignite, cool and weigh a porcelain crucible, brush in the weighed sample and burn at a low temperature, using a burner, or place the uncovered crucible in a muffle furnace heated to about 700°. The crucible should be kept at dull redness until the carbon is all consumed and the ash becomes nearly white. A gray or black appearance of the residue indicates the presence of unburned carbon but a red tint may be given by iron oxide normally present. Cool in a desiccator, weigh and calculate the per cent of ash.

Mineral Analysis.—The solution of the ash in hydrochloric acid is diluted to 250 cc and the mineral constituents determined as described under soil analysis, beginning on page 256. This analysis involves a considerable expenditure of time and it is rarely useful, except in the solution of certain research problems.

Crude Fat or Ether Extract.—The nature of the material obtained by extracting feeds with ether varies according to the nature of the feed. Grains and other seeds yield nearly pure fat, while in fibrous materials many compounds, such as waxes, resins and chlorophyl, also are extracted by the ether.

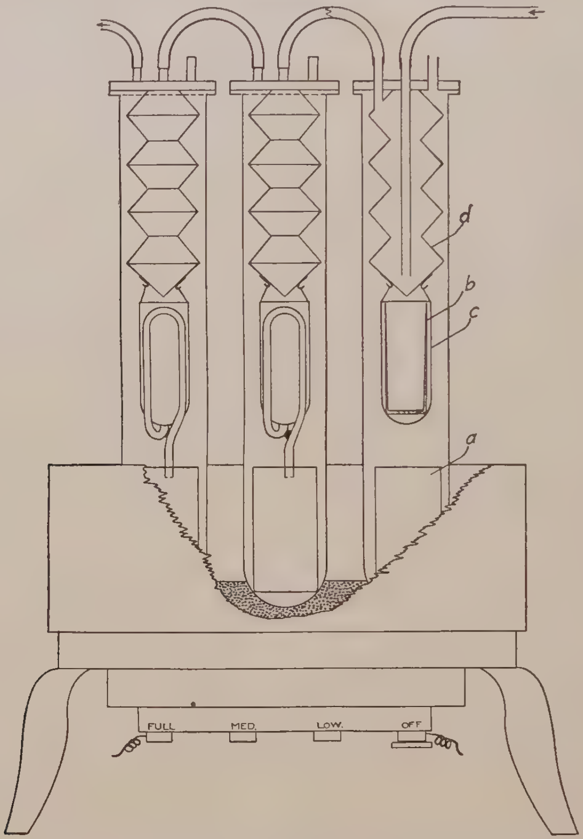

Fig. 41.—Apparatus for extraction by volatile solvents.

When there is present in the feed a considerable amount of soluble carbohydrates, such as starch or sugars, and a relatively small amount of fat, as in wheat and rye, it is best to dissolve out these substances with water before attempting to extract the fat. It is quite essential that samples to be extracted be thoroughly dried and that the ether be free from alcohol and water as otherwise various substances, soluble in water or alcohol (salts, sugars and amids) would be extracted. Certain other fat solvents have been used, such as benzol, gasoline and carbon tetrachloride, but none has been found to be quite as satisfactory as dry ether.

One form of assembled extraction apparatus is shown in Fig. 41. Any number of separate pieces may be assembled upon one heater. In Fig. 41 the third extractor is shown in section. Ether (or other volatile solvent) is placed in the weighed cup *a* where it boils and the condensed vapor from the condenser *d* falls to the sample in the porous cup *b*. As the solvent fills the siphon cup *c* to the level of the siphon bend, the cup automatically empties into *a*, below. This process repeats itself indefinitely.

Determination of Crude Fat.—Wash commercial ether by shaking in a separatory funnel with two or three successive portions of water and drawing off and discarding the latter. Add solid sodium or potassium hydroxide and let stand until most of the water has been abstracted from the ether. Decant into a dry bottle, add small pieces of cleaned metallic sodium or sodium wire, freshly extruded from a sodium press, and let stand until there is no further evolution of hydrogen. Keep the ether, thus dehydrated, over metallic sodium in lightly stoppered bottles.

The sample is thoroughly dried at 100° in an alundum cup or fat-free paper capsule (or the sample used for the moisture determination is taken), then placed in an extracting tube and sufficient ether, as above prepared, is added to the weighed cup *a* of Fig. 41 (previously cleaned, dried and weighed) to enable continuous extraction to proceed automatically. The alundum cup is a porous vessel, cylindrical in shape, and convenient to use because it is easily cleaned by burning, so that it may be used repeatedly. It is suitable to use also in fiber filtration, as it permits weighing and burning of the fiber without removal to another vessel. The porous alundum cup, grade R. A. 98, permits rapid filtering and washing.

Extract the sample for sixteen hours, saving the residue for the fiber determination (page 148), then remove the cup *a* and allow the ether to evaporate, or place the cup in a special apparatus for distilling and recovering the ether. Dry at 100° for 30 minutes, cool in a desiccator and weigh. Repeat the drying for 30-minute periods until the weight is constant. From the difference between the weights before and after extraction, calculate the per cent of crude fat.

Crude Fiber.—The so-called "crude fiber" is a mixture of substances which make up the framework of a plant. It is composed of cellulose, part of the hemicellulose and lignin of the cell walls. Lignin also is a collective name applied to the "incrusting substances" formed with cellulose as the plant matures.

Determination of Crude Fiber.—Prepare the following solutions:

(*a*) *Sulphuric Acid,* 1.25 per cent (0.255 normal) as determined by titration against a fifth-normal base.

(*b*) *Sodium Hydroxide,* 1.25 per cent (0.3125 normal). This solution should be practically free from sodium carbonate. Use sodium hydroxide sticks that have been purified by alcohol. Titrate against a standard acid and adjust.

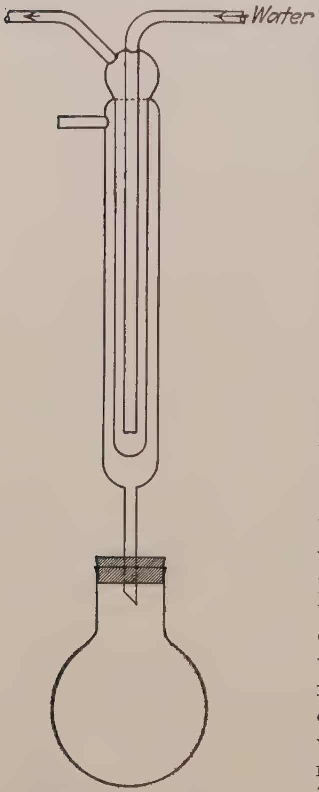

Fig. 42.—Apparatus for crude fiber determination.

Use the residue from the ether extraction, page 147, or extract a fresh dry sample (2 gm) with ether, using the apparatus already described. Rinse the residue into a 250-cc wide mouth flask connected with a return condenser (Fig. 42), then add 200 cc of sulphuric acid (*a*), previously heated to boiling. Boil gently for 30 minutes. After this time remove the residue from the flask and filter the liquid through an alundum cup, using suction. Wash the cup and contents until free from acid. Wash the residue from the cup back into the flask with 200 cc of boiling sodium hydroxide (*b*) and boil for 30 minutes. Filter through an alundum cup as before and wash free from base. The cup is dried at 110° to constant weight, then the residue is burned and the per cent of fiber calculated from the loss in weight. A linen filter may be used instead of an alundum cup, in which case rinse the washed fiber into a flat platinum dish by means of a stream of water; evaporate to dryness on a steam bath, dry to constant weight at 110°, weigh, burn the organic matter and weigh again. The loss in weight is crude fiber. If a weighed paper is used instead of alundum or linen, weigh in a weighing bottle. In any case the crude fiber after drying to constant weight at 110° must be burned and the loss of weight determined.

Optional Method.[1]—Proceed as above until the acid extraction is completed. Neutralize the sulphuric acid without filtration, using 10-per cent sodium hydroxide and phenolphthalein. Add 200 cc of 2.6-per cent solution of boiling sodium hydroxide, make the volume up to 425 cc and boil for 30 minutes. Filter through an alundum cup and wash with hot water until neutral to phenolphthalein. Dry the residue in an oven for 3 hours at 110°, place in a desiccator, cool and weigh. Ignite as in the ash determination, cool and weigh again. The loss in weight represents fiber. Calculate the per cent of crude fiber in the sample.

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[1] *Ohio Exp. Sta. Bull.,* **255** (1913).

Crude Protein.—"Crude protein," is a conventional term, embracing all forms of plant nitrogenous bodies except nitrates. The latter are not usually found in feeding stuffs. There is no good direct method for determining protein and either pure or "crude" protein is calculated from the per cent of nitrogen. Since plant proteins contain about 16 per cent of nitrogen, the nitrogen per cent is multiplied by 6.25 $\left(=\frac{100}{16}\right)$ to convert it to the approximate corresponding per cent of protein. The nitrogen content varies for proteins of different classes. In the determination of milk protein 6.38 is the factor used, but nitrogen found in grasses and fruits is partly in the form of amids and has a lower conversion factor because of somewhat higher per cent.

Nitrogen.—From the above discussion it will be seen that the determination of protein rests upon the nitrogen determination. The Kjeldahl process for this determination consists in digesting the organic material with boiling concentrated sulphuric acid until complete decomposition has been effected. The exact course of the reactions cannot be traced but the carbon and hydrogen are completely oxidized and nitrogen is converted into ammonia, which immediately combines with sulphuric acid and remains as ammonium sulphate. The completion of decomposition is insured by the final addition of a small amount of potassium permanganate. The solution is then diluted with water, an excess of sodium hydroxide is added and the resultant ammonia is distilled into a measured quantity of standard acid solution, the excess of which is then titrated by a standard base.

Digestion.—The digestion with sulphuric acid is best accomplished in a pear-shaped flask with a long neck, like that shown in Fig. 43. The concentrated sulphuric acid of commerce boils at temperatures ranging from 210° to 340°, according to the per cent of water contained in it. Such a temperature is high enough above that of the surrounding air to permit condensation of nearly all of the vapor without the use of a water condenser, the long neck of the digestion flask serving for this purpose. It is convenient to distill from the flask in which digestion is accomplished, in which case the capacity of the flask should be 500 cc. The digestion must be performed under a hood or some other provision must be made for carrying away the fumes. An excellent arrangement for this purpose is a lead pipe, 6 inches in diameter and with holes in the side so that the necks of a number of digestion flasks may be inserted with the flask in an inclined position. The end of the lead pipe leads to a chimney.

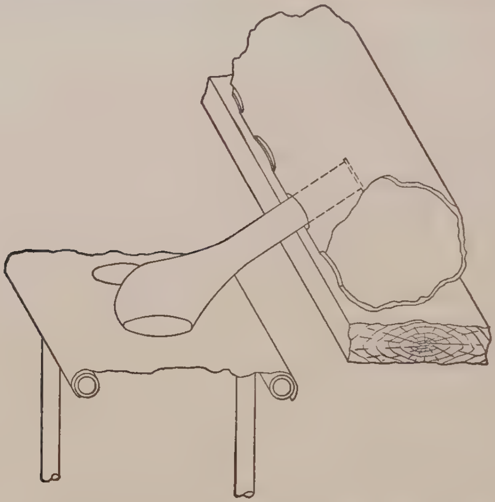

FIG. 43.—Kjeldahl flask, stand and lead pipe ventilator.

Catalytic Agents.—The addition of oxides or salts of mercury, copper or iron to the mixture of the organic material and sulphuric acid considerably accelerates the reactions that occur during digestion. The action is of a catalytic nature and depends upon the capability of the metal of existing in more than one state of oxidation. The metal is thus alternately reduced by organic matter and oxidized by sulphuric acid, somewhat as follows:

$$2HgSO_4 \rightarrow Hg_2SO_4 + SO_3 + O. \qquad (1)$$

The nascent oxygen thus formed attacks the organic matter and mercurous sulphate is immediately reoxidized:

$$Hg_2SO_4 + 2H_2SO_4 \rightarrow 2HgSO_4 + 2H_2O + SO_2. \qquad (2)$$

Of the three metals named, mercury serves well because its salts are colorless and they do not obscure the end point of the oxidation. It is necessary in this case to precipitate the mercury by the addition of potassium sulphide, before distillation, in order to prevent the formation of mercurammonium compounds which later are not readily decomposed by sodium hydroxide. Copper sulphate as a catalyst is often preferred because it serves as an indicator when sodium hydroxide is added, a deep blue solution being formed when the solution becomes basic.

Prevention of Bumping.—During the distillation of ammonia, after the addition of excess of sodium hydroxide, there is usually a tendency toward bumping. In order to prevent this, granular zinc or pumice stone may be used. An excellent substitute is a small amount (about 0.5 gm) of crushed porcelain from which the dust has been removed by sifting.

Blank.—Sulphuric acid nearly always contains a small amount of ammonium sulphate. Distilled water which has been exposed to laboratory air also may contain a small quantity of ammonium hydroxide. In order to make the proper correction for the ammonia that will be derived from the reagents a "blank" determination must be made, omitting the sample of feed but carrying out the operations exactly as in the real determination. In this case cane sugar is added to reduce possible traces of nitrates existing in the reagents, as they would be reduced by the organic matter of the feed.

Determination of Organic Nitrogen (of Crude Protein): *Kjeldahl Method.*—Prepare the following reagents:

(*a*) *Hydrochloric or Sulphuric Acid Solution, Fifth-normal.*—Standardize against pure sodium carbonate as directed on page 58, making the necessary changes in weight of carbonate to account for the different normality of the acid here used. The standardization of these acids by weighing silver chloride or barium sulphate (the official methods) is not to be recommended because chlorides and sulphates, respectively, are nearly always to be found in the acids. These would give high values for the acid content, so determined.

(*b*) *Sodium Hydroxide or Potassium Hydroxide Solution, Fifth-normal.*—Standardize by titration against the acid (*a*), using methyl red as indicator.

(*c*) *Sulphuric Acid.*—The concentrated acid of the laboratory, specific gravity 1.84, as nearly as possible free from nitrates and ammonium salts.

(*d*) *Metallic Mercury, Mercuric Oxide or Cupric Sulphate.*—Mercuric oxide should be that prepared in the wet way but not from mercuric nitrate.

(*e*) *Potassium Sulphide Solution.*—Dissolve at the rate of 40 gm for each liter of solution. Commercial potassium sulphide is used. This solution is not required unless mercury or mercuric oxide is to be used as the catalyzer.

(*f*) *Sodium Hydroxide Solution.*—A saturated solution (55 gm per 100 cc of water), free from nitrates and containing as little carbonate as possible.

(*g*) *Methyl Red Solution.*—Dissolve 1 gm of methyl red in 100 cc of 95-per cent alcohol. This is the solution ordinarily used in volumetric analysis. Add very dilute acid or base to make exactly neutral.

If the approximate per cent of nitrogen in the sample is known, calculate the weight that will yield ammonia equivalent to about 35 cc of the standard acid. If nothing is known of the nitrogen content use about 2 gm of sample. (For this method the sample must contain no nitrates, nitrites, or nitro-compounds. This is ordinarily true with feeds.) Place two weighed samples in 500-cc Kjeldahl digestion flasks, holding the latter in a vertical position to prevent the sample from sticking to the sides of the neck, which should be dry. Weigh 1 gm of sugar into each of two other flasks and treat the same as the feed sample. Add about 0.7 gm of mercuric oxide or of mercury, or 0.3 gm of copper sulphate, also 25 cc of concentrated sulphuric acid. Incline the flask in a hood or with the neck inserted into a lead-pipe ventilator and heat gently until the violence of the reactions has moderated, then gradually raise the temperature until the acid is boiling. The flask may be heated without protection by a gauze if it is of Pyrex or similar resistance glass and if it is placed over a hole in a stand of sheet iron in such a manner that the flame cannot come into contact with the sides of the flask above the liquid.

Digest by gently boiling until the solution is nearly colorless (blue if copper sulphate has been used). This may occur after a short time or the digestion may require several hours. Finally remove the flame and at once drop into the flask small quantities of powdered potassium permanganate until the solution acquires a green or purple tint which persists after shaking. Allow the flask to stand until cool. (*Do not cool under a tap.*) Carefully add 200 cc of distilled water and mix by rotating the flask. Add about 0.5 gm of crushed porcelain and 25 cc of potassium sulphide solution (*e*), shaking as the latter is added. (If cupric sulphate has been used as a catalyzer, omit the potassium sulphide solution.)

Have the connections with a tin condenser ready and have 50 cc of standard acid measured into a 250-cc flask into which the delivery tube (of glass) dips. Most laboratories in which much work of this kind is done will be equipped with a special form of apparatus for carrying on several distillations at once. Such an apparatus as is shown in Fig. 44 will be found convenient for individual work. The flask should be in a vertical position and some kind of trap should be used to prevent spray from being carried over by the steam. The delivery tube should be capable of being detached from the condenser for the purpose of cleaning and rinsing it. The entire condenser must be thoroughly rinsed before each distillation, to insure freedom from basic solutions.

Pour 50 cc of saturated sodium hydroxide solution (*f*) down the inclined flask in such a way that mixing does not occur. Immediately connect with the condenser, carefully mix the contents of the flask by shaking gently, then distill into the standard acid until about 150 cc of distillate has been collected. It sometimes happens that a considerable excess of sulphuric acid has been used in order to hasten a difficult digestion, or that the sodium hydroxide solution is not saturated. The consequence is that the solution still contains an excess of acid when ready for distillation. This will not be the case if the directions have been carefully followed but the addition of a drop of phenolphthalein to the solution will serve to indicate the fact. (It should be remembered, however, that a concentrated solution of a base soon decolorizes phenolphthalein and this action may be mistaken for an indication of an excess of acid.) If copper sulphate has been used as an accelerator a deep blue color will indicate the presence of sufficient sodium hydroxide.

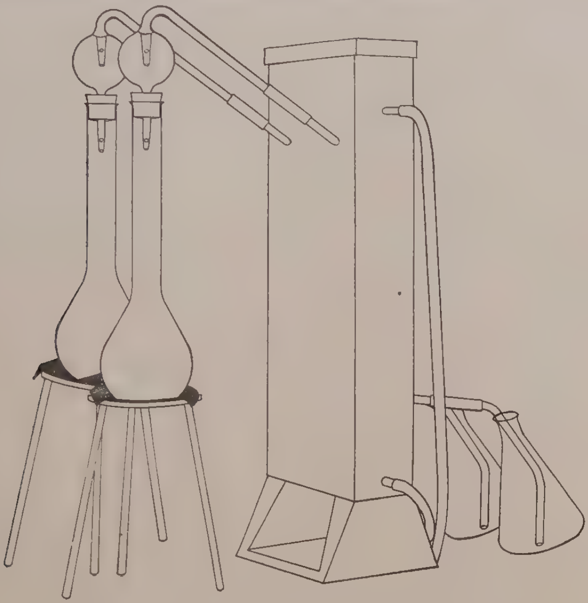

FIG. 44.—Apparatus for ammonia distillations.

When the distillation is finished lower the receiving flask until the delivery tube is above the liquid, then remove the flame. Disconnect the delivery tube from the condenser and rinse inside and outside, allowing the rinsings to run into the flask. Add enough methyl red to tint the solution then titrate with standard base. Subtract the excess of acid thus indicated and calculate the per cent of nitrogen in the sample, making proper correction for any nitrogen found in the reagents by the blank determination with sugar. Multiply the result by 6.25 and express as crude protein.

Gunning Method.—It was observed by Gunning[1] that in the ordinary Kjeldahl process the water produced by the oxidation of organic matter dilutes the sulphuric acid and retards its action. Gunning proposed the addition of potassium sulphate, thus forming acid sulphates which lose water much more readily than the hydrates of sulphuric acid so that the solution is easily concentrated by boiling. The potassium sulphate also raises the boiling point of the acid and a higher temperature is attained during the digestion. A mixture of one part of potassium sulphate and two parts of sulphuric acid is heated together and finally allowed to cool. This mixture is measured into the digestion flask, where the digestion is performed as in the Kjeldahl process except that no mercury is added and, consequently, no potassium sulphide is needed before the distillation. In the method as now carried out the required amounts of potassium sulphate and sulphuric acid are added directly to the flask without preliminary heating. Copper sulphate may be used as an accelerator.

Determination of Organic Nitrogen: *Gunning Method.*—Calculate the weight of sample required, as in the Kjeldahl method, and weigh this amount into digestion flasks. Add to the sample in the digestion flask 10 gm of powdered potassium sulphate and 15 to 25 cc of concentrated sulphuric acid. Digest as in the Kjeldahl process except that 0.3 gm of copper sulphate is used instead of mercury, mercuric oxide or potassium permanganate. When the solution is clear blue, cool, dilute and conduct the distillation as in the Kjeldahl process, omitting, however, the potassium sulphide solution. Make a blank determination as in the Kjeldahl process. Calculate the per cent of nitrogen in the sample.

Kjeldahl-Gunning-Arnold Method.—This method of digestion combines the accelerating action of mercury salts, potassium sulphate and cupric sulphate. Otherwise the method is not essentially different from those already described. It is not applicable to materials containing nitrates.

Determination of Nitrogen: *Kjeldahl-Gunning-Arnold Method.*—Digest the usual amount of sample with 10 gm of potassium sulphate, 1 gm of cupric sulphate, 1 gm of mercury or mercuric oxide and 25 cc of concentrated sulphuric acid. Heat gently until frothing ceases, then boil the mixture briskly and continue the digestion until the solution is colorless or nearly so or until oxidation is complete. Cool, dilute with about 200 cc of water, and add 50 cc of potassium sulphide solution. Make basic and distill as in the Kjeldahl method.

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[1] *Z. anal. Chem.*, **28**, 188 (1889).

Non-protein (Amid) Nitrogen.—The non-protein forms of nitrogen compounds are usually soluble in water and they are not precipitated by copper hydroxide. This fact is utilized in effecting a separation of the amids from true proteins as the latter form an insoluble compound with copper hydroxide, which may be separated from the amid by filtration. The amount of protein nitrogen in the residue is determined by the Kjeldahl method. This per cent, subtracted from that of total nitrogen, gives the amid nitrogen.

Determination of Protein Nitrogen.—Prepare *cupric hydroxide* as follows: Dissolve 100 gm of pure cupric sulphate in 5 liters of water, add 2.5 cc of glycerol and then 10-per cent sodium hydroxide until the liquid is just basic. Allow the precipitate of cupric hydroxide to settle and decant off the supernatant liquid. Add distilled water containing 5 per cent of glycerol, decant and continue to wash the precipitate by decantation with this glycerol solution until the washings are no longer basic to phenolphthalein. Rub the cupric hydroxide precipitate in a mortar with enough water containing 10 per cent of glycerol to make a uniform gelatinous mass capable of being measured with a pipette. Calculate the weight of cupric hydroxide in 1 cc of the mixture.

Place a 1-gm sample of the feed in a beaker and add 100 cc of water. If the feed is high in alkaline phosphates (as are seeds and oil meals) 3 cc of a pure saturated potassium alum solution (free from ammonia) should be added to avoid any solution of the protein-copper precipitate. Heat slowly to boiling and add sufficient cupric hydroxide reagent to contain about 1 gm of cupric hydroxide. Stir thoroughly and filter after the liquid has cooled. Wash with cold water, place the paper and washed residue in a Kjeldahl digestion flask and determine the amount of protein nitrogen, as already directed for nitrogen of crude protein.

Amid Nitrogen.—Calculate the per cent of amid nitrogen by deducting the protein nitrogen from the total nitrogen of the sample.

Carbohydrates.—Carbohydrates are found in vegetable feeds in variable quantities. In corn, they range from 70 per cent in the grain to 16 per cent in the stalks. Their food value depends to a considerable extent upon the degree of solubility as a result of mild hydrolysis and enzyme action. The ones that are immediately soluble in water are the most readily digestible. Sugars and dextrins are the most important of these. Starch is next in importance as it is easily made soluble in digestive processes by hydrolytic action. Other groups such as the hemicelluloses (examples of which are the pentosans, galactans and pectins) are made soluble with more difficulty and they are therefore less valuable as foods. That portion of the carbohydrates which does not yield soluble forms on hydrolysis is practically worthless for feeding purposes. It is chiefly cellulose and from the analysis it is reported as "crude fiber."

The pentosans are widely distributed in the vegetable kingdom, being present in the seeds, roots and leaves of all plants. One of the most common of the pentosans is gum Arabic, which occurs intimately associated with the other plant constituents. The galactans also are widely distributed in plants and they occur chemically combined with the pentosans in the plant. Agar-agar is one of the most common of the galactans. It yields galactose upon hydrolysis, while pentosans yield pentose sugars when similarly treated.

Analytical Methods.—Carbohydrates in foods and feeds are determined (*a*) by direct acid hydrolysis and subsequent determination of the reducing sugar thus formed, (*b*) by hydrolysis of starch by diastase, thus forming dextrins, maltose and glucose, or (*c*) by difference, deducting from 100 the sum of the per cents of crude protein, crude fat, ash, crude fiber and moisture. This difference is reported as "nitrogen-free extract."

True starch cannot be determined accurately by direct hydrolysis with acids because other polysaccharides, such as gums, pentosans and galactans, are hydrolyzed at the same time, yielding reducing sugars which are determined along with those that are derived from starch. The type reaction of hydrolysis is as represented in the equation:

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6.$$

A separation from these hydrolyzable materials may be made by first digesting with the enzyme diastase, from malt extract, then washing out the soluble carbohydrates and hydrolyzing them to glucose by boiling with dilute acids. By this procedure only the true starches are affected by the enzyme and a series of compounds of simpler structure are formed. A large number of dextrins are formed as intermediate products. Some of these (erythrodextrins) give a red color with iodine while others (acroödextrins) give no color. Under the influence of the acid these dextrins finally yield maltose, a sugar having the same molecular weight as sucrose.

Polarimetric methods are not well suited to the determination of the carbohydrates in feeds, because of the relatively small amounts usually occurring in such materials. Greater reliance is placed upon chemical methods, such as those here to be described.

Reducing Sugars.—"Reducing sugars" are those that have the power of reducing the copper from an alkaline solution of copper tartrate to cuprous oxide, Cu_2O. Dextrose, levulose, maltose and invert sugar are examples of common reducing sugars while sucrose is a non-reducing sugar.

It has already been stated that reducing sugars may either be present in the original material or they may be formed as a result of hydrolysis of other carbohydrates, such as starch or sucrose. Therefore the determination of original reducing sugars may conveniently be combined with that of sucrose.

Calculation of Reducing Sugars from the Weight of Cuprous Oxide.—When the weight of cuprous oxide is used as a basis for calculating weights of sugars, the method of reducing and precipitating must be definitely standardized as the formation of cuprous oxide does not proceed according to an absolutely definite and constant reaction, depending not only upon the kinds and amounts of reducing sugars present but also upon the temperature and concentration of the solution and upon the length of time it is heated. Tables have been prepared for different sugars, giving the amount of cuprous oxide reduced by each under specified conditions. These are given in Table VII, pages 160 and 161.

Methods for Determining the Reduced Cuprous Oxide.—A number of methods are in use for the determination of the cuprous oxide reduced by the sugars. Three of these will be described.

In method (*a*) the solution is filtered through a Gooch crucible, the cuprous oxide then being dried and weighed as such or ignited and weighed as cupric oxide. Direct weighing is suitable only in the case of solutions of pure sugars. Molasses and syrups usually contain colloidal organic matter which cannot be washed out of the precipitate. It is then necessary to ignite in air, when cuprous oxide is oxidized to cupric oxide and organic matter is destroyed by oxidation.

Method (*b*) is an approximate volumetric one, differing from method (*a*) in that a standard copper sulphate solution is used, whose sugar equivalent is known.

In method (*c*) the cuprous oxide is removed and redissolved and the copper is determined volumetrically by the "iodide" method. Potassium iodide and acetic acid are added, cuprous oxide being precipitated and iodine liberated:

$$2Cu(C_2H_3O_2)_2 + 4KI \rightarrow 2CuI + 4KC_2H_3O_2 + I_2.$$

The free iodine is titrated with standard sodium thiosulphate and the copper equivalent to it calculated.

It is also practicable to dissolve the cuprous oxide and to determine the copper by electrolysis or by any other standard gravimetric or volumetric method.

Asbestos.—Since this is to be used as a filtering medium for strongly basic solutions it must be prepared with special reference to removing base-soluble materials. The amphibole variety is required, as serpentine asbestos is too easily soluble.

Determination of Sucrose and Reducing Sugars.—Prepare the following materials:

(*a*) *Fehling's Solution* (1).—Dissolve 34.639 gm of pure crystals of copper sulphate in distilled water and dilute to 500 cc. Filter, if not clear, through asbestos.

(*b*) *Fehling's Solution* (2).—Dissolve .173 gm of sodium potassium tartrate ("Rochelle salts") and 50 gm of sodium hydroxide in water and dilute to 500 cc. Allow the solution to stand for two days and filter through asbestos, if not clear.

(*c*) *Neutral Lead Acetate.*—Prepare a saturated solution of lead acetate (the normal salt). This is made by warming 50 gm of lead acetate with 100 cc of water until the salt is dissolved, then cooling to room temperature.

(*d*) *Asbestos.*—Digest the fiber for three days with dilute hydrochloric acid. Wash free from acid in a large funnel fitted with a perforated porcelain plate, then digest for a similar period with 10-per cent sodium hydroxide solution. Drain away this solution and then treat for two or three hours with alkaline tartrate solution similar to solution (*b*), above described. Wash practically free from base and then digest for several hours with dilute nitric acid. Finally wash free from acid and shake the material to a pulp with distilled water. The prepared material is now to be used as any other asbestos for forming Gooch filters.

Extraction of Sugars from the Feed.—Place 12 gm of the material in a 250-cc round flask and, if the substance has an acid reaction, add 2 gm of calcium carbonate. Add 150 cc of 50-per cent alcohol (volume) and boil on the steam bath for one hour, using a reflux condenser. Cool and allow the mixture to stand for several hours. Rinse into a 250-cc volumetric flask with 95-per cent alcohol which is not acid to phenolphthalein and dilute to the mark with this alcohol. Mix thoroughly, allow to settle, transfer 200 cc to a beaker with a pipette and evaporate on a steam bath to a volume of about 20 cc. (Do not evaporate to dryness, a little alcohol in the residue doing no harm.)

Clarification.—Transfer to a 100-cc graduated flask and rinse the beaker thoroughly with water, adding the rinsings to the contents of the flask. Add enough saturated neutral lead acetate solution (*c*) to produce a flocculent precipitate, shake thoroughly and allow to stand for 15 minutes. Dilute to the mark on the flask, mix thoroughly and filter most of the solution through a dry paper, rejecting the first 5 cc of filtrate. Add sufficient anhydrous sodium carbonate to the filtrate to precipitate all of the lead, again filter through a dry paper and test the filtrate with a little more sodium carbonate, in order to be sure that all of the lead has been removed.

This solution will serve for the determination of both sucrose and reducing sugars.

Since the insoluble material of grain or cattle food occupies some space in the flask as originally made up, it is necessary to correct for this volume. Results of a large number of determinations on various materials have shown the average volume of 12 gm of material to be 9 cc, and therefore to obtain the true amount of sugars present all results must be multiplied by the factor 0.964 $\left(=\frac{250-9}{250}\right)$. If the sample weight was not 12 gm (±0.5 gm) the factor should be modified accordingly.

Reducing Sugars.—Measure 25 cc of the copper sulphate solution (*a*) and 25 cc of the alkaline tartrate solution (*b*) into a 400-cc beaker. Add 25 cc of water and 20 cc of the sugar solution already prepared. Cover the beakers with watch glasses and heat on an asbestos mat at such a rate that boiling begins in 4 minutes. Continue the boiling for exactly 2 minutes. Filter through Gooch crucibles (weighed if method (*a*), below, is to be followed) immediately after heating and wash thoroughly with hot water (about 60°.) From this point proceed by one of the following methods:

(*a*) *Gravimetric Method.*—The Gooch filters must be dried, ignited, cooled and weighed before filtration. After filtration dry the crucible and contents, then place in a muffle furnace which is heated to redness (about 700°) and heat for 15 minutes. Cool and weigh and from the weight of cupric oxide find that of dextrose from Table VII. Multiply by 6.025$\left(=0.964 \times \frac{100}{20} \times \frac{250}{200}\right)$ and calculate the corresponding per cent of dextrose in the 12-gm feed sample, reporting as "reducing sugars."

TABLE VII.—MUNSON AND WALKER'S TABLE FOR CALCULATING DEXTROSE, INVERT SUGAR ALONE, INVERT SUGAR IN THE PRESENCE OF SUCROSE, LACTOSE, AND MALTOSE (All weights are given in milligrams)

Cuprous oxide	Copper	Dextrose	Invert sugar	Invert sugar and sucrose		Lactose		Maltose		Cuprous oxide
				0.4 gm total sugar	2 gm total sugar	Anhydrous	Crystalline	Anhydrous	Crystalline	
10	8.9	4.0	4.5	1.6	.....	3.8	4.0	5.9	6.2	10
15	13.3	6.2	6.7	3.9	.....	7.1	7.5	9.9	10.4	15
20	17.8	8.3	8.9	6.1	.....	10.4	10.9	13.8	14.6	20
25	22.2	10.5	11.2	8.4	.....	13.7	14.4	17.8	18.7	25
30	26.6	12.6	13.4	10.7	4.3	16.9	17.8	21.8	22.9	30
35	31.1	14.8	15.6	12.9	6.5	20.2	21.3	25.7	27.1	35
40	35.5	16.9	17.8	15.2	8.8	23.5	24.8	29.7	31.3	40
45	40.0	19.1	20.1	17.5	11.1	26.8	28.2	33.7	35.4	45
50	44.4	21.3	22.3	19.7	13.4	30.1	31.7	37.6	39.6	50
55	48.9	23.5	24.6	22.0	15.7	33.4	35.1	41.6	43.8	55
60	53.3	25.6	26.8	24.3	18.0	36.7	38.6	45.6	48.0	60
65	57.7	27.8	29.1	26.6	20.3	40.0	42.1	49.5	52.1	65
70	62.2	30.0	31.3	28.9	22.6	43.3	45.5	53.5	56.3	70
75	66.6	32.2	33.6	31.2	24.9	46.6	49.0	57.5	60.5	75
80	71.1	34.4	35.9	33.5	27.3	49.9	52.5	61.4	64.6	80
85	75.5	36.7	38.2	35.8	29.6	53.1	56.0	65.4	68.8	85
90	79.9	38.9	40.4	38.2	31.9	56.4	59.4	69.3	73.0	90
95	84.4	41.1	42.7	40.5	34.2	59.7	62.9	73.3	77.2	95
100	88.8	43.3	45.0	42.8	36.6	63.0	66.4	77.3	81.3	100
105	93.3	45.5	47.3	45.2	38.9	66.4	69.8	81.2	85.5	105
110	97.7	47.8	49.6	47.5	41.3	69.7	73.3	85.2	89.7	110
115	102.2	50.0	51.9	49.8	43.6	73.0	76.8	89.2	93.9	115
120	106.6	52.3	54.3	52.2	46.0	76.3	80.3	93.1	98.0	120
125	111.0	54.5	56.6	54.5	48.3	79.6	83.8	97.1	102.2	125
130	115.5	56.8	58.9	56.9	50.7	82.9	87.3	101.0	106.4	130
135	119.9	59.0	61.2	59.3	53.1	86.2	90.8	105.0	110.5	135
140	124.4	61.3	63.6	61.6	55.5	89.5	94.2	109.0	114.7	140
145	128.8	63.6	65.9	64.0	57.8	92.8	97.7	112.9	118.9	145
150	133.2	65.9	68.3	66.4	60.2	96.1	101.2	116.9	123.0	150
155	137.7	68.2	70.6	68.8	62.6	99.5	104.7	120.8	127.2	155
160	142.1	70.4	73.0	71.2	65.0	102.8	108.2	124.8	131.4	160
165	146.6	72.8	75.3	73.6	67.4	106.1	111.7	128.8	135.5	165
170	151.0	75.1	77.7	76.0	69.8	109.4	115.2	132.7	139.7	170
175	155.5	77.4	80.1	78.4	72.2	112.8	118.7	136.7	143.9	175
180	159.9	79.7	82.5	80.8	74.6	116.1	122.2	140.6	148.0	180
185	164.3	82.0	84.9	83.2	77.1	119.4	125.7	144.6	152.2	185
190	168.8	84.3	87.2	85.6	79.5	122.7	129.2	148.6	156.4	190
195	173.2	86.7	89.6	88.0	81.9	126.1	132.7	152.5	160.5	195
200	177.7	89.0	92.0	90.5	84.4	129.4	136.2	156.5	164.7	200
205	182.1	91.4	94.5	92.9	86.8	132.7	139.7	160.4	168.9	205
210	186.5	93.7	96.9	95.4	89.2	136.0	143.2	164.4	173.0	210
215	191.0	96.1	99.3	97.8	91.7	139.4	146.7	168.3	177.2	215
220	195.4	98.4	101.7	100.3	94.2	142.7	150.2	172.3	181.4	220
225	199.9	100.8	104.2	102.7	96.6	146.0	153.7	176.2	185.5	225
230	204.3	103.2	106.6	105.2	99.1	149.4	157.2	180.2	189.7	230
235	208.7	105.6	109.1	107.7	101.6	152.7	160.7	184.2	193.8	235
240	213.2	108.0	111.5	110.1	104.0	156.1	164.3	188.1	198.0	240
245	217.6	110.4	114.0	112.6	106.5	159.4	167.8	192.1	202.2	245
250	222.1	112.8	116.4	115.1	109.0	162.7	171.3	196.0	206.3	250
255	226.5	115.2	118.9	117.6	111.5	166.1	174.8	200.0	210.5	255

TABLE VII.—(*Continued*)

Cuprous oxide	Copper	Dextrose	Invert sugar	Invert sugar and sucrose		Lactose		Maltose		Cuprous oxide
				0.4 gm total sugar	2 gm total sugar	Anhydrous	crystalline	Anhydrous	crystalline	
260	231.0	117.6	121.4	120.1	114.0	169.4	178.3	203.9	214.7	260
265	235.4	120.0	123.9	122.6	116.5	172.8	181.9	207.9	218.8	265
270	239.8	122.5	126.4	125.1	119.0	176.1	185.4	211.8	223.0	270
275	244.3	124.9	128.9	127.7	121.6	179.5	188.9	215.8	227.1	275
280	248.7	127.3	131.4	130.2	124.1	182.8	192.4	219.7	231.3	280
285	253 2	129.8	133.9	132.7	126.6	186.2	196.0	223.7	235.5	285
290	257.6	132.3	136.4	135.3	129.2	189.5	199.5	227.6	239.6	290
295	262.0	134.7	138.9	137.8	131.7	192.9	203.0	231.6	243.8	295
300	266.5	137.2	141.5	140.4	134.2	196.2	206.6	235.5	247.9	300
305	270.9	139.7	144.0	142.9	136.8	199.6	210.1	239.5	252.1	305
310	275.4	142.2	146.6	145.5	139.4	203.0	213.7	243.5	256.3	310
315	279.8	144.7	149.1	148.1	141.9	206.3	217.2	247.4	260.4	315
320	284.2	147.2	151.7	150.7	144.5	209.7	220.7	251.3	264.6	320
325	288.7	149.7	154.3	153.2	147.1	213.1	224.3	255.3	268.7	325
330	293.1	152.2	156.8	155.8	149.7	216.4	227.8	259.3	272.9	330
335	297.6	154.7	159.4	158.4	152.3	219.8	231.4	263.2	277.0	335
340	302.0	157.3	162.0	161.0	154.8	223.2	234.9	267.1	281.2	340
345	306.5	159.8	164.6	163.7	157.5	226.5	238.5	271.1	285.4	345
350	310.9	162.4	167.2	166.3	160.1	229.9	242.0	275.0	289.5	350
355	315.3	164.9	169.8	168.9	162.7	233.3	245.6	279.0	293.7	355
360	319.8	167.5	172.5	171.5	165.3	236.7	249.1	282.9	297.8	360
365	324.2	170.1	175.1	174.2	167.9	240.0	252.7	286.9	302.0	365
370	328.7	172.7	177.7	176.8	170.6	243.4	256.2	290.8	306.1	370
375	333.1	175.3	180.4	179.5	173.2	246.8	259.8	294.8	310.3	375
380	337.5	177.9	183.0	182.1	175.9	250.2	263.4	298.7	314.5	380
385	342.0	180.5	185.7	184.8	178.5	253.6	266.9	302.7	318.6	385
390	346.4	183.1	188.4	187.5	181.2	256.9	270.5	306.6	322.8	390
395	350.9	185.7	191.0	190.2	183.9	260.3	274.0	310.6	326.9	395
400	355.3	188.4	193.7	192.9	186.5	263.7	277.6	314.5	331.1	400
405	359.7	191.0	196.4	195.6	189.2	267.1	281.1	318.5	335.2	405
410	364.2	193.7	199.1	198.3	191.9	270.5	284.7	322.4	339.4	410
415	368.6	196.3	201.8	201.0	194.6	273.9	288.3	326.3	343.5	415
420	373.1	199.0	204.6	203.7	197.3	277.3	291.9	330.3	347.7	420
425	377.5	201.7	207.3	206.5	200.0	280.7	295.4	334.2	351.8	425
430	382.0	204.4	210.0	209.2	202.7	284.1	299.0	338.2	356.0	430
435	386.4	207.1	212.8	212.0	205.5	287.5	302.6	342.1	360.1	435
440	390.8	209.8	215.5	214.7	208.2	290.9	306.2	346.1	364.3	440
445	395.3	212.5	218.3	217.5	211.0	294.2	309.7	350.0	368.4	445
450	399.7	215.2	221.1	220.2	213.7	297.6	313.3	353.9	372.6	450
455	404.2	218.0	223.9	223.0	216.5	301.1	316.9	357.9	376.7	455
460	408.6	220.7	226.7	225.8	219.2	304.5	320.5	361.8	380.9	460
465	413 0	223.5	229.5	228.6	222.0	307.9	324.1	365.8	385.0	465
470	417.5	226.2	232.3	231.4	224.8	311.3	327.7	369.7	389.2	470
475	421.9	229.0	235.1	234.2	227.6	314.7	331.3	373.7	393.3	475
480	426.4	231.8	237.9	237.1	230.3	318.1	334.8	377.6	397.5	480
485	430.8	234.6	240.8	239.9	233.2	321.5	338.4	381.5	401.6	485
490	435.3	237.4	243.6	242.7	236.0	324.9	342.0	385.5	405.8	490

(*b*) *Iodide Method.*—Sodium thiosulphate solution is prepared and standardized as follows. Dissolve 19 gm of pure crystals of sodium thiosulphate and dilute to 1000 cc with recently boiled and cooled distilled water. Mix well. One cubic centimeter should then be equivalent to about 0.005 gm of copper. Weigh in duplicate about 0.2 gm of pure copper foil, place in 250-cc Erlenmeyer flasks and dissolve the copper by adding 10 cc of a mixture of equal parts of water and concentrated nitric acid. Boil until red fumes have been expelled, add 40 cc of water and 5 cc of saturated bromine water, mix and boil until the bromine vapor has disappeared. Add 7 cc of ammonium hydroxide and boil again to expel excess of ammonia, but not far enough to cause a precipitate. Add 4 cc of glacial acetic acid (or 40 cc of 10-per cent acid), cool to room temperature and add 10 cc of 30-per cent potassium iodide solution. Immediately titrate with sodium thiosulphate until the solution bearing the white precipitate shows only a faint yellow tinge and then add 1 cc of starch indicator. (The starch indicator is made by mixing 1 gm of starch with 1 cc of cold water, pouring into this 100 cc of boiling water and boiling for a minute. This solution should be made fresh each day, as required.) Continue the titration with sodium thiosulphate until the blue color is discharged. Calculate the weight of copper equivalent to 1 cc of the solution.

Drop into the Gooch crucible containing the cuprous oxide, 5 cc of warm nitric acid (1:1) and cover the crucible. Collect the filtrate in a 250-cc flask. Wash the crucible once or twice with hot water. Pour 5 cc of bromine water into the crucible, then wash with 50 cc of hot water. Boil filtrate and washings to expel bromine, then proceed from this point as directed for standardizing sodium thiosulphate solution. Calculate the weight of copper present, from which the corresponding weight of dextrose can be obtained by reference to table VII. Multiply by 6.025 (see page 159) and calculate the per cent of reducing sugars in the feed sample.

Approximate Volumetric Method.—Prepare:

Standard Invert Sugar Solution.[1]—Dissolve 4.75 gm of pure sucrose in 75 cc of water, add 5 cc of concentrated hydrochloric acid and let stand at a temperature not below 20° for 24 hours, or for 10 hours if the temperature is above 25°. The solution should not be heated. Neutralize the acid with 5-per cent sodium hydroxide solution (using methyl orange), dilute to 1000 cc in a volumetric flask and mix well. Ten cubic centimeters of this solution contain 0.050 gm of invert sugar and it should reduce about 5 cc of the Fehling's copper solution. Standardize as follows:

Pipette 5 cc of each of Fehling's solutions (*a*) and (*b*) (page 158) into a small casserole or beaker and add 10 cc of water. Heat to boiling and add, from a burette, 9 cc of the standard invert sugar solution and boil for 2 minutes. This should reduce nearly all of the copper to cuprous oxide, removing all but a faint blue color. Continue to add small portions of the invert sugar solution, boiling after each addition. When the end is nearly reached and the amount of sugar solution to be added can no longer be judged by the color of the solution, remove about 1 cc of the liquid and filter rapidly into a small porcelain crucible or on a test plate; acidify with 10-per cent acetic acid and test for copper with a 5-per cent potassium ferrocyanide solution. After the end point has been reached calculate the invert sugar equivalent of the cupric sulphate solution.

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[1] For a discussion of the common sugars and of the process of "inversion" of sucrose, see page 131, Part II.

Determine the reducing sugars of the solution containing the extract from the feed by adding it to the Fehling's solution in the manner described for the standard invert sugar solution. In this case the approximate sugar content is not known and the first trial may show that too much was added. If so, make another trial, modifying the volume of sugar solution to be added.

Calculate the weight of reducing sugar as dextrose which, of course, has the same equivalent weight as does invert sugar. Multiply by $\frac{120.5}{v}$ $\left(= 0.964 \times \frac{250}{200} \times \frac{100}{v}\right)$, where v = volume of sugar solution used, and calculate the per cent in the original 12 gm of sample.

Determination of Sucrose.—Pipette 25 cc of the clarified sugar solution from the feed sample (page 159) into a 100-cc volumetric flask, add a few drops of methyl orange, neutralize with dilute hydrochloric acid and then add 5 cc of concentrated hydrochloric acid and allow the inversion to proceed at 20° for 24 hours. Neutralize with sodium carbonate, then dilute to the mark with water, filter if necessary and determine reducing sugars in 50 cc of the solution by any of the methods described for reducing sugars. Multiply by 9.64 $\left(=0.964 \times \frac{250}{200} \times \frac{100}{25} \times \frac{100}{50}\right)$ to obtain the weight of sugar in the original 12 gm of sample. Calculate the per cent. Subtract the per cent of reducing sugars before inversion from the percent of total reducing sugar after inversion, both being calculated as invert sugar, and multiply the remainder by 0.95 $\left(\frac{C_{12}H_{22}O_{11}}{2C_6H_{12}O_6} = \frac{342}{360} = 0.95\right)$ to obtain the per cent of sucrose.

Starch: *Diastase Hydrolysis.*—When starch is gelatinized by boiling with water it is possible to convert it to maltose and dextrin by treating it with either ptyalin or malt diastase, these enzymes accelerating the hydrolysis:

$$(C_6H_{10}O_5)_x + \frac{x}{2}H_2O \rightarrow \frac{x}{2}C_{12}H_{22}O_{11}.$$

(Taka-diastase,[1] if available, is more convenient to use, no blank determination being required with malt extract.) The enzymes thus introduced have no action on other carbohydrates present. Starches from the different grains are not acted upon with equal vigor by diastase so it is necessary to test with iodine solution to determine whether the conversion has been completed.

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[1] *J. Agr. Sci.*, **11**, 9 (1921).

After hydrolysis of the starch by the enzyme, the resulting maltose and dextrin may be further hydrolyzed to dextrose under the influence of acid as follows:

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{Dextrose}}{2C_6H_{12}O_6}.$$

The dextrose so produced is then determined by methods already described for reducing sugars.

The work should be so planned that the determination can be carried through without delay. If an interruption is necessary after the completion of the enzyme action, fermentation should be prevented by the addition of 0.2 gm of salicylic acid.

Determination of Starch: *Diastase Method.*—Prepare malt extract as follows:

Grind about 10 gm of malt and add to it 200 cc of water. Allow it to digest at the temperature of the room for about three hours, with occasional shaking. Filter. Determine the weight of reducing sugars in 40 cc of the extract, after treatment with hydrochloric acid as described below for the feed.

Extract on a hardened filter 5 gm of the dry material, very finely ground, with five successive portions of 10 cc of ether. Wash with 150 cc of 10-per cent alcohol and then with a little 95-per cent alcohol. This removes all fatty material and sugars. Place the residue in a beaker with 50 cc of water, immerse the beaker in boiling water and stir constantly for 15 minutes or until all the starch is gelatinized. Cool to 55°, add 20 cc of malt extract and maintain at this temperature by placing in a water bath for an hour. Heat again to boiling for a few minutes, cool to 55°, add 20 cc of malt extract and maintain at this temperature for an hour or until particles of the residue treated with iodine show no blue color upon microscopic examination. Cool, make up directly to 250 cc and filter through a dry paper. Place 200 cc of the filtrate in a flask with 20 cc of 25 per cent hydrochloric acid (specific gravity 1.125). Connect with a reflux condenser and heat in a boiling water bath for 2.5 hours. Cool, nearly neutralize with sodium hydroxide solution, finish the neutralization with sodium carbonate solution (using methyl orange) and make up to 500 cc in a volumetric flask. Mix the solution well, pour through a dry filter and determine the dextrose in 50 cc as directed on page 159. Conduct a blank determination upon 40 cc of the malt extract by hydrolyzing with acid, with subsequent determination of copper reduced, and correct the weight of copper reduced by the feed solution accordingly. The weight of the dextrose obtained multiplied by 0.93 gives the weight of starch. Calculate the per cent.

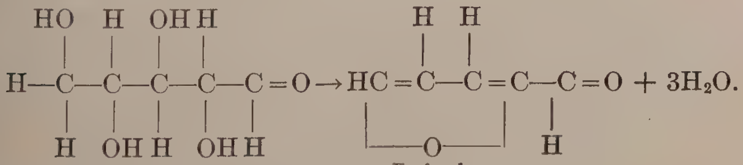
Direct Acid Hydrolysis.—Members of the starch group comprised in the "nitrogen free extract" are often determined by direct acid hydrolysis. When the mixed feed is boiled with acid, after most of the fat has been removed, the starch and some of the pentosans are hydrolyzed to reducing sugars. It is due to the pentosans that these results, considered as starch, are too high when compared with the amount obtained by the diastase method just described.

Determination of Starch: *Direct Acid Hydrolysis.*—Stir a quantity of the sample, representing 2.5 to 3 gm of the dry material, in a beaker with 50 cc of cold water for an hour. Transfer to a filter and wash with 250 cc of cold water. Heat the insoluble residue with 200 cc of water and 20 cc of 25-per cent hydrochloric acid (specific gravity 1.125) and boil for 2.5 hours, in a flask provided with a reflux condenser. Cool, rinse into a 250-cc volumetric flask and nearly neutralize with sodium hydroxide, using methyl orange. Dilute to 250 cc mix and filter, and determine the dextrose in 50 cc of the filtrate as directed on page 159, omitting the addition of water just before mixing with Fehling's solution. The weight of the dextrose obtained multiplied by 0.93 gives the weight of starch.

The factor 0.90 is the theoretical ratio between starch and glucose but, according to Noyes and other investigators,[1] the factor 0.93 more nearly represents the analytical ratio.

Arabin, Xylan and the Pentosans.—These are compounds of unknown constitution but they all yield pentoses (aldehyde sugars containing five carbon atoms) upon hydrolysis under the influence of hydrochloric acid. Arabin and xylan are constituents of the plant gums. Arabin may be obtained from gum Arabic, while xylan is found in many woods, in straw and in corn cobs. Lignin is one of the most common of the pentosans. It occurs with cellulose in wood, straw, bran and similar materials. It will thus be seen that all of these substances will be probable constituents of the rougher materials of the sort to be found in animal feeds.

The pentoses which are formed by hydrolysis of the compounds already mentioned are further converted into the aldehyde furfural, upon distillation with hydrochloric acid. The type reaction is as follows:



A pentose

Furfural

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[1] *J. Am. Chem. Soc.*, **26**, 266 (1904).

In the analytical method, furfural is produced by hydrolysis and distillation with hydrochloric acid. Phloroglucin, an aromatic alcohol, $C_6H_3(OH)_3$, is added and this precipitates furfural phloroglucide:

$$C_5H_4O_2 + C_6H_3(OH)_3 \rightarrow C_{11}H_8O_4 + H_2O$$

From the weight of furfural phloroglucide the corresponding weight of pentosans, may be found by referring to Krober's table, found on page 167.

Determination of Pentosans and Allied Substances.—Prepare the following reagents:

Phloroglucin.—Test the purity of the laboratory supply by dissolving a small amount in a few drops of acetic anhydride, heating almost to boiling and adding a few drops of concentrated sulphuric acid. If more than a faint violet color appears the phloroglucin contains diresorcin and it must then be purified. For this purpose heat 11 gm of phloroglucin with 300 cc of 12-per cent hydrochloric acid (specific gravity 1.06), adding the phloroglucin very gradually. Continue heating and stirring until solution is nearly complete. Pour the hot solution into 1200 cc of hydrochloric acid of the same concentration. Allow to stand for several days, to permit the diresorcin to crystallize. Filter just before using.

Aniline Acetate Paper.—This is prepared by mixing aniline and water in equal volumes, then adding glacial acetic acid until the mixture is clear. Moisten filter paper with the solution.

Place 2 to 5 gm of feed in a 250-cc distilling flask which is fitted with a separatory funnel and which is connected with a condenser. Add 100 cc of 12-per cent hydrochloric acid (1.06 specific gravity) and several pieces of pumice stone, dropped in while hot. Heat over a wire gauze at such a rate that about 30 cc will distill over in 10 minutes, passing the distillate through a small filter paper into a 500-cc volumetric flask. Add 30 cc of 12-per cent hydrochloric acid to the flask through the separatory funnel. Continue this process of distilling and replacing the distillate by hydrochloric acid until the distillate amounts to about 360 cc and until a few drops give no red or pink color to aniline acetate paper.

Gradually add to the total distillate an amount of pure phloroglucin about double the furfural estimated to be present. (Consult the instructor.) It will be observed there are several color changes taking place, the solution becoming yellow, then green and finally an almost black precipitate appears. The solution is diluted to 400 cc with 12-per cent hydrochloric acid and allowed to stand for 12 hours. Test the solution with aniline acetate paper to see if precipitation of furfural has been complete, a red color developing if any furfural remains in solution. Filter the precipitate through a dried and weighed Gooch crucible. Wash with 150 cc of water (retaining some water in the Gooch crucible until the last, during the washing) and dry for 4 hours at 100°. Cover the crucible, cool and weigh rapidly.

The weight of pentosans cannot be calculated accurately from that of phloroglucide by use of a constant factor which has been derived from the theoretical equation, because of variation in the composition of the furfural phloroglucide, according to the proportion of furfural present.

TABLE VIII.—KROBER'S TABLE FOR DETERMINING PENTOSES, PENTOSANS AND RELATED SUBSTANCES

Furfural-phloroglucide	Furfural	Arabinose	Arabin	Xylose	Xylan	Pentose	Pentosan
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
0.035	0.0209	0.0446	0.0393	0.0370	0.0326	0.0408	0.0359
0.040	0.0235	0.0501	0.0441	0.0416	0.0366	0.0459	0.0404
0.045	0.0260	0.0556	0.0490	0.0462	0.0406	0.0509	0.0448
0.050	0.0286	0.0611	0.0538	0.0507	0.0446	0.0559	0.0492
0.055	0.0312	0.0666	0.0586	0.0553	0.0486	0.0610	0.0537
0.060	0.0338	0.0721	0.0634	0.0598	0.0526	0.0660	0.0581
0.065	0.0364	0.0776	0.0683	0.0644	0.0567	0.0710	0.0625
0.070	0.0390	0.0831	0.0731	0.0690	0.0607	0.0761	0.0670
0.075	0.0416	0.0886	0.0780	0.0736	0.0647	0.0811	0.0714
0.080	0.0442	0.0941	0.0828	0.0781	0.0687	0.0861	0.0758
0.085	0.0468	0.0996	0.0877	0.0827	0.0727	0.0912	0.0803
0.090	0.0494	0.1051	0.0925	0.0872	0.0767	0.0962	0.0847
0.095	0.0520	0.1106	0.0974	0.0918	0.0808	0.1012	0.0891
0.100	0.0546	0.1161	0.1022	0.0964	0.0848	0.1063	0.0935
0.105	0.0572	0.1215	0.1070	0.1010	0.0888	0.1113	0.0979
0.110	0.0598	0.1270	0.1118	0.1055	0.0928	0.1163	0.1023
0.115	0.0624	0.1325	0.1166	0.1101	0.0968	0.1213	0.1067
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
0.125	0.0676	0.1435	0.1263	0.1192	0.1049	0.1314	0.1156
0.130	0.0702	0.1490	0.1311	0.1237	0.1089	0.1364	0.1201
0.135	0.0728	0.1545	0.1360	0.1283	0.1129	0.1414	0.1244
0.140	0.0754	0.1600	0.1408	0.1328	0.1169	0.1464	0.1288
0.145	0.0780	0.1655	0.1457	0.1374	0.1209	0.1515	0.1333
0.150	0.0805	0.1710	0.1505	0.1419	0.1249	0.1565	0.1377
0.155	0.0831	0.1765	0.1554	0.1465	0.1289	0.1615	0.1421
0.160	0.0857	0.1820	0.1602	0.1510	0.1329	0.1665	0.1465
0.165	0.0883	0.1875	0.1650	0.1556	0.1369	0.1716	0.1510
0.170	0.0909	0.1930	0.1698	0.1601	0.1409	0.1766	0.1554
0.175	0.0935	0.1985	0.1746	0.1647	0.1449	0.1816	0.1598
0.180	0.0961	0.2039	0.1794	0.1692	0.1489	0.1866	0.1642
0.185	0.0987	0.2093	0.1842	0.1734	0.1529	0.1916	0.1686
0.190	0.1013	0.2147	0.1889	0.1783	0.1569	0.1965	0.1729
0.195	0.1039	0.2201	0.1937	0.1829	0.1609	0.2015	0.1773
0.200	0.1065	0.2255	0.1984	0.1874	0.1649	0.2065	0.1817
0.205	0.1090	0.2309	0.2032	0.1920	0.1689	0.2115	0.1861
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
0.215	0.1142	0.2417	0.2127	0.2011	0.1770	0.2214	0.1948
0.220	0.1168	0.2471	0.2174	0.2057	0.1810	0.2264	0.1992
0 225	0.1194	0.2525	0.2222	0.2102	0.1850	0.2314	0.2037
0.230	0.1220	0.2579	0.2270	0.2148	0.1890	0.2364	0.2081
0.235	0.1245	0.2633	0.2318	0.2193	0.1930	0.2413	0.2124
0.240	0.1271	0.2687	0.2365	0.2239	0.1970	0.2463	0.2168
0.245	0.1297	0.2741	0.2413	0.2284	0.2010	0.2513	0.2212
0.250	0.1323	0.2795	0.2460	0.2330	0.2050	0.2563	0.2256
0.255	0.1349	0.2849	0.2508	0.2375	0.2090	0.2612	0.2299
0.260	0.1374	0.2903	0.2555	0.2420	0.2130	0.2662	0.2342
0.265	0.1400	0.2957	0.2603	0.2465	0.2170	0.2711	0.2385
0.270	0.1426	0.3011	0.2650	0.2511	0.2210	0.2761	0.2429
0.275	0.1452	0.3065	0.2698	0.2556	0.2250	0.2811	0.2473
0.280	0.1478	0.3199	0.2745	0.2602	0.2290	0.2681	0.2517
0.285	0.1504	0.3173	0.2793	0.2647	0.2330	0.2910	0.2561
0.290	0.1529	0.3227	0.2840	0.2693	0.2370	0.2960	0.2605
0.295	0.1555	0.3281	0.2887	0.2738	0.2410	0.3010	0.2649
0.300	0.1581	0.3335	0.2935	0.2784	0.2450	0.3060	0.2693

Krober's table, page 167, gives the weights of furfural, pentoses and pentosans for weights of phloroglucide between 0.03 and 0.30 gm. For weights less than 0.03 gm, use the following formulas:

$$\text{Furfural} = 0.5170\,(a + 0.0052), \tag{1}$$
$$\text{Pentoses} = 1.0170\,(a + 0.0052), \tag{2}$$
$$\text{Pentosans} = 0.8949\,(a + 0.0052), \tag{3}$$

where a = weight of phloroglucide and 0.0052 represents weight of phloroglucide soluble in the 400 cc of acid solution.

Galactans.—These are substances of unknown constitution which, like the pentosans, are widely distributed in the vegetable kingdom. Agar-agar is one of the important members of this group. Another is the principal carbohydrate of the soybean. When the galactans are hydrolyzed by acids they yield galactose, a sugar having the same empirical formula as dextrose, and nitric acid further converts this into mucic acid, $C_4H_4(OH)_4(COOH)_2$.

The galactans are of considerable importance in feeds. They are said to be utilizable to the extent of 50 per cent by herbivorous animals, but agar-agar only 8 to 27 per cent by man.[1]

Determination of Galactans.—Prepare reagents as follows:

(*a*) *Ammonium Carbonate Solution.*—Dissolve 2 gm of ammonium carbonate in 38 cc of water and add 2 cc of concentrated ammonium hydroxide.

(*b*) *Nitric Acid.*—Prepare 250 cc of nitric acid, specific gravity 1.15.

Extract an accurately weighed sample of about 2.5 gm on a hardened paper, with five successive portions of 10 cc each of ether, place the insoluble residue in a beaker, about 5.5 cm in diameter and 7 cm deep, together with 60 cc of nitric acid (*b*) and evaporate the solution to exactly one-third of its initial volume in a water bath whose temperature is 94° to 96°. After standing for 24 hours add 10 cc of water and allow to stand another 24 hours. The mucic acid has, in the meantime, crystallized but it is mixed with other material only partly oxidized by the nitric acid. Filter, wash with 30 cc of water to remove as much of the nitric acid as possible and replace the filter and contents in the beaker. Add 30 cc of ammonium carbonate solution (*a*) and heat the mixture on a water bath at 80° for 15 minutes, with constant stirring.

The ammonium carbonate reacts with the mucic acid, forming soluble ammonium mucate. Wash the filter paper and contents several times with hot water by decantation, passing the washings through the filter paper, to which finally transfer the material and thoroughly wash. Evaporate the filtrate to dryness on a water bath, avoiding unnecessary heating (which causes decomposition), add 5 cc of nitric acid (*b*), stir the mixture thoroughly and allow to stand for 30 minutes. The nitric acid decomposes ammonium mucate, precipitating mucic acid; collect this on a weighed Gooch or alundum crucible, wash with 10 to 15 cc of water, then with 60 cc of alcohol, and finally several times with ether. Dry at 100° for 3 hours, cool and weigh. Multiply the weight of mucic acid by 1.33, which gives galactose, or by 1.197, which gives galactan. Calculate the per cent of galactan in the feed.

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[1] SAIKI, *J. Biol. Chem.*, **2,** 251 (1906).

CHAPTER X

SAPONIFIABLE OILS, FATS AND WAXES

Composition.—The chief constituents of animal and vegetable oils are esters derived from fatty acids and glycerol, a triatomic alcohol. Of the former the most important are palmitic, stearic and oleic acids, the first two being saturated, the last an unsaturated acid. The glycerides of these acids are respectively known as palmitin, stearin and olein and they have the following composition:

$$\underset{\text{Palmitin}}{C_3H_5(C_{16}H_{31}O_2)_3}, \quad \underset{\text{Stearin}}{C_3H_5(C_{18}H_{35}O_2)_3}, \quad \underset{\text{Olein}}{C_3H_5(C_{18}H_{33}O_2)_3}.$$

In addition to these are esters of higher alcohols other than glycerine and of other saturated and unsaturated fatty acids, also in certain cases small amounts of free higher alcohols. The chief differences in properties of different oils are caused by variations in the proportions of the constituent esters. Vegetable oils contain much palmitin while stearin predominates in animal oils. The more liquid oils contain more olein and esters of acids having smaller molecular weights.

The animal and vegetable oils and fats are thus in a class quite distinct from that of mineral oils, the latter being mixtures of various saturated and unsaturated hydrocarbons, not saponifiable, as distinguished from the saponifiable esters of the former class.

Waxes.—The true waxes differ chemically from the oils and fats in that they are not glycerides but are esters of mono- or diatomic alcohols with the higher fatty acids. These alcohols are either aliphatic or aromatic. Following are some examples of such esters: Cetyl palmitate, derived from palmitic acid and cetyl alcohol, $C_{16}H_{33}OH$; this is the chief constituent of spermaceti. Ceryl palmitate, the chief constituent of opium wax, is derived from palmitic acid and ceryl alcohol, $C_{27}H_{55}OH$. Myricyl palmitate occurs in beeswax. It is an ester of palmitic acid and myricyl alcohol, $C_{30}H_{61}OH$. Ceryl cerotate is the chief constituent of Chinese wax. It is an ester of cerotic acid, $C_{25}H_{51}COOH$, and ceryl alcohol. The most important aromatic alcohols occurring in waxes are the isomeric alcohols cholesterol and phytosterol, $C_{26}H_{43}OH$. These are found as esters of palmitic, stearic and oleic acids.

Separation and Identification.—Notwithstanding the differences in composition the task of separating and determining the per cent of different oils in a mixture is a difficult and sometimes impossible one, because of the fact that the same general compounds constitute the greater proportion of all fats and oils. The chemist must usually be satisfied if he can recognize single oils or, with the nature of a single oil known, determine the approximate extent and nature of adulteration. The differences in molecular weight and degree of saturation, the presence and per cent of free alcohols or acids and the occasional occurrence of traces of unusual substances, characteristic of certain oils, constitute the bases of the tests used in the effort to identify an oil. The examination becomes therefore not an analysis, in the usual sense, but a series of tests applied in order to gain information regarding the identity of a pure oil and, so far as is possible, the composition of a mixture. Certain physical and chemical "constants" are determined and compared with the constants obtained from examination of oils of known purity. The chief obstacle to the use of such figures lies in the fact that, for a given kind of oil they are actually variable within certain limits. These limits may be very narrow, but it sometimes happens that the ranges for two or more oils overlap. Thus olive oil from Italy is not chemically identical with olive oil from California. The soil, climate, variety of plant and method of expressing from the olive have their influence upon the properties of the various glycerides and other substances present in the oil. It is only when the ranges of variation for different oils do not overlap that it is easy to determine the identity of a single oil, although it usually happens that while overlapping occurs with a single constant it does not occur with others.

The significance of the various constants and their methods of determination will be described.

Specific Gravity.—In a general way the specific gravity of oils increases with the per cent of (*a*) glycerides of unsaturated acids, (*b*) glycerides of soluble acids and (*c*) free fatty acids. Old oils also usually have higher specific gravities than the normal, on account of oxidation. The specific gravity of the waxes and of solid fats is usually higher than of liquid oils. These rules do not hold in all cases and the determination of specific gravity, like that of the other constants of oils, is made for comparing with recorded data for the purpose of identification more often than for throwing light upon the chemical constitution of oils of known purity.

The principles underlying the modes of expression and determination of specific gravity have been discussed on pages 94 to 102, Part II. Unfortunately there has been a great lack of uniformity in selecting conditions and modes of expression for specific gravities of oils as they are recorded in the literature. Temperatures of 15.5°, 17.5°, 20°, 25°, 40°, 60°, 100° and others are commonly used. In favor of the higher temperatures it may be said that the fats and waxes are all liquid at these temperatures so that determinations may readily be made. It has been found[1] that a fair degree of approximation may be made in correcting the specific gravity to another temperature by using the coefficient 0.0007 as the change for each Centigrade degree. This is the average value for a considerable number of oils between temperatures of 15.5° and 98°. Of course this does not remedy the lack of uniformity of expression, noted above.

For the determination use a picnometer, a Westphal balance or an accurately calibrated hydrometer. If a Westphal balance is used the displacement of the plummet in pure boiled water should be accurately determined at the temperature at which the balance is to be used. The thermometer in the plummet should be compared with a standard thermometer. The picnometer method is recommended.

Determination of Specific Gravity of Oils at $\frac{20^\circ}{20^\circ}$.—Use a 25-cc specific gravity bottle (picnometer). Clean with chromic acid, followed by distilled water, then rinse with alcohol and dry in an oven at 100°. Cool in the balance case (in which the air should be at a temperature not above 20°) and weigh. Fill with distilled water which has been recently boiled to expel dissolved gases and cooled to a few degrees below 20°. Insert the stopper and nearly immerse the stoppered bottle in a bath of distilled water which is kept at exactly 20°. After 30 minutes take off the drop of water from the tip of the stopper, remove the bottle and wipe perfectly dry with a clean towel but without warming the bottle to above 20°. Place in the balance case and weigh after 15 minutes. Calculate the weight of contained water.

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[1] Wright, *J. Soc. Chem. Ind.*, **26**, 513 (1907).

Empty the bottle and dry inside and out, then fill with oil and manipulate as before, calculating the weight of contained oil. This weight divided by the weight of contained water gives the specific gravity of the oil at $\frac{20°}{20°}$.

If the specific gravity has been determined at any other temperature or if it is desired to calculate the specific gravity at any temperature from the determination at 20°, use the following formula:

$$G = G' + 0.0007\ (t' - t),\ \text{where}$$

G = specific gravity at temperature t,

G' = specific gravity at temperature t′.

Determination of Specific Gravity at $\frac{20°}{4°}$.—Multiply the specific gravity at $\frac{20°}{20°}$ by 0.99897, which is the density of water at 20°. The product is the specific gravity of the oil at $\frac{20°}{4°}$. (See page 94.)

Determination at the Temperature of Boiling Water.—Fill a 25-cc picnometer, dried and weighed as above described, with freshly boiled hot water. Nearly immerse in a bath of briskly boiling water and leave for 30 minutes, replacing evaporated water with boiling distilled water. Insert the stopper, previously heated to 100°, remove the picnometer from the bath, wipe dry, cool to room temperature and weigh. Calculate the weight of contained water.

Fill the flask, dried at 100°, with the dry, hot, freshly filtered fat or oil, which must be entirely free from air bubbles. Keep in the boiling water bath for 30 minutes then insert the stopper, which has been heated to 100°, wipe dry, cool to room temperature and weigh. Calculate the weight of contained oil and from this and the weight of water contained at boiling temperature calculate the specific gravity of the oil at the temperature of boiling water.

This determination is necessarily less accurate than the one at 20°, on account of the difficulty involved in keeping the bath at any constant temperature. Superheating may easily occur with distilled water and less pure water may have a boiling point above 100°. Variation in barometric pressure will also change the temperature of the bath so that it becomes necessary to carry out both parts of the experiment at the same atmospheric pressure. However the determination is sanctioned and has been made official by the Association of Official Agricultural Chemists.

The specific gravity at any temperature other than 20 may be determined by the method outlined for this temperature or it may be calculated from the determination at this temperature, using the formula given above. It should be understood that the figure desired for purposes of identification is the specific gravity at the temperature for which data may be found in the literature.

Index of Refraction.—A discussion of the underlying theory and of the determination of index of refraction is found on pages 113 to 120, Part II.

The measurement of index of refraction is a valuable addition to the list of tests for oils. While not in all cases characteristic it will frequently serve to distinguish between certain possibilities when other tests are not conclusive. The refractive index increases with (*a*) increasing molecular weight of the combined acids and (*b*) increasing unsaturation. If free fatty acids are present in an oil the refractive index will be lower than the normal value for the oil. In consequence of the latter fact one may expect to find abnormally low indices for old or rancid fats or oils.

The selection of standard temperatures for the determination is highly desirable in order to make comparison data useful. Temperatures of 20° for oils and 40° or 60° for fats and waxes are suitable in most cases. For calculating the index of refraction at any temperature from experimental results at another temperature the following formula may be used:

$$R = R' + 0.000365\,(t' - t),$$

where R and R' indicate indices of refraction at temperatures t and t', respectively. The coefficient 0.000365 is the average change of index for 1° for a large number of common oils.

The index of refraction of oils is conveniently determined by use of any of the standard instruments, such as the Abbé, Pulfrich, Zeiss butyro-refractometer or the immersion refractometer. Of those named the Abbé instrument is probably the most generally useful because it may be used with liquids covering a wide range of refractive indices and because it does not require the use of monochromatic light. The principles underlying the use of this and other instruments are discussed on pages 115 to 120, Part II.

Determination of Index of Refraction by Means of the Abbé Refractometer.—Set up the instrument in front of a window or any artificial light source, noting, that monochromatic light is not essential. Connect a constant temperature apparatus furnished with the instrument and adjust the flow of water and the height of the flame until the desired temperature (20° for oils, 40° or higher for fats or waxes) is attained. Open the prism so that the lower half is in a horizontal position and place two or three drops of oil or melted fat or wax upon it, using a glass rod or pipette but avoiding scratching the prisms. Quickly close and lock the system, allow time for the temperature to become constant and then adjust the compensator and focus until the line of division of the field is sharply defined and bring this to the cross hairs. Read the index of refraction upon the scale.

Clean the prisms by applying a mixture of equal volumes of alcohol and ether, using a tuft of absorbent cotton.

Melting Point of Fats.—From the fact that fats are mixtures and not pure compounds, it will be seen that they cannot have definite and sharp melting points. The observation will therefore be a somewhat arbitrary one. The following is Wiley's method.

Determination.—Prepare discs of fat as follows: Allow the melted and filtered fat to fall a distance of about 20 cm, from a dropping tube to a piece of ice or to the surface of cold mercury. The discs thus formed should be 1 to 1.5 cm in diameter and they should weigh about 200 mg. Since a recently melted and solidified fat does not have its normal melting point the discs should stand two to three hours before testing.

Prepare an alcohol-water mixture of graduated density, as follows: Boil, separately, water and 95-per cent alcohol for ten minutes to remove dissolved gases. While still hot pour the water into a 20 cm test tube until it is almost half full. Nearly fill the tube with the hot alcohol, pouring down the side of the inclined tube, to avoid too much mixing.

Place the test tube containing the alcohol-water mixture in a tall beaker containing ice water, until cold. Drop the disc of fat into the tube and it will at once sink to a point where the density of the mixture is exactly equal to its own. Lower an accurate thermometer, graduated to tenths, into the test tube until the bulb is just above the disc, stirring very gently. Slowly heat the water in the beaker, stirring constantly with an air blast or mechanical stirrer.

When the temperature of the alcohol-water mixture has risen to a point about 6° below the melting point of the fat the disc will begin to shrivel and roll into an irregular mass. Now lower the thermometer until the fat particle is even with the center of the bulb. Rotate the thermometer gently and regulate the temperature so that about 10 minutes is required for the last increment of 2°. As soon as the fat becomes a spherical globule read the thermometer. This serves as a preliminary determination of melting point.

Remove the tube from the bath and place in the latter a second tube of alcohol and water. The latter, having been cooled in ice water, is sufficiently low in temperature to cool the bath to the desired point. Add another disc of fat and regulate the temperature so as to reach a maximum of 1.5° above the melting point as already determined. Run a third determination, which should agree closely with the second.

The disc of fat should not be allowed to touch the side of the tube, in any determination.

Iodine Absorption Number.—The iodine absorption number is the per cent of halogen, expressed as iodine, absorbed by the fat or oil when subjected to the action of a halogen solution under specified conditions. The absorption takes place because of the presence of glycerides of unsaturated acids, which contain double or triple bonded carbon atoms.

This action is analogous to the absorption of oxygen. In the latter case saturated oxygen compounds are formed, often hard and resinous in nature. Absorption of oxygen from the air in this way is known as "drying," although the term is misapplied, since no real drying occurs. The determination of halogen absorption number is, in a general way, a measure of drying properties and it serves as a distinction between the somewhat arbitrary classes of drying, semi-drying and non-drying oils.

Of the unsaturated acids whose glycerides commonly occur in fats or oils the following important members may be mentioned:

Oleic Acid, $C_{18}H_{34}O_2$.—The unsaturated character of this acid is indicated by the formula

$$CH_3(CH_2)_7CH=CH(CH_2)_7COOH.$$

Olein, the triglyceride of this acid, occurs to some extent in all oils and fats, but especially in the former. The empirical formula of the triglyceride is

$$C_3H_5(C_{18}H_{33}O_2)_3.$$

Olein is liquid at ordinary temperatures and its presence in oils is responsible, in a large number of cases, for their liquid character.

Oleic acid will absorb two atoms of bromine, iodine or chlorine, or one molecule of iodine monochloride or monobromide, the double bonded carbon atoms thus becoming saturated. Similarly, either oleic acid or olein might be expected to absorb oxygen and to give drying properties to a fat or oil but this action does not take place readily and most of the oils of pronounced drying properties are found to contain considerable quantities of simple or mixed glycerides of linolic or linolenic acids, more highly unsaturated compounds than oleic acid.

Linolic Acid, $C_{18}H_{32}O_2$, contains two pairs of doubly linked carbon atoms:

$$CH_3(CH_2)_4CH=CH\cdot CH_2\cdot CH=CH(CH_2)_7COOH.$$

This acid will absorb four atoms of halogen or two atoms of oxygen. It gives marked drying properties to oils, linolin being abundant in linseed, soybean and poppy seed oils.

Linolenic Acid, $C_{18}H_{30}O_2$, probably to be represented as

$$CH_3\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot(CH_2)_7COOH.$$

This acid possesses three sets of double bonds and will absorb six halogen atoms or three oxygen atoms. It occurs as simple or mixed glycerides in linseed oil and, together with linolic acid, plays the most important part in the hardening or "drying" of this oil when it is exposed to the air. An isomer, isolinolenic acid, also occurs as a constituent of the glycerides of drying oils.

Ricinoleic Acid, $C_{18}H_{34}O_3$, is hydroxyoleic acid and, like oleic acid itself, contains only one pair of doubly linked carbon atoms. It will not readily absorb oxygen from the air and it does not impart drying properties to an oil. It is, however, an important constituent of castor oil and will be mentioned later, in the discussion of acetyl value.

The five acids named above serve to illustrate the principle that only those unsaturated acids which contain more than one pair of doubly bonded carbon atoms are important from the standpoint of drying. Also an interesting, although perhaps unexpected fact is that *trebly* linked carbon atoms do not, under ordinary conditions, absorb halogens or oxygen to the point of complete saturation, only two atoms of halogen or one of oxygen adding to each such pair.

Solvent.—Absorption of halogen by oil cannot readily take place unless there is present some solvent which can dissolve both oil and halogen. The halogen solution earliest used for this purpose was of iodine and mercuric chloride in alcohol. This has been almost entirely replaced by a solution of either iodine monobromide or iodine monochloride in glacial acetic acid. The monobromide solution was proposed by Hanus, that of monochloride by Wijs. As the former is somewhat more easily prepared its preparation and use will be described.

The following solutions will be required for the determination of iodine number:

(*a*) *Potassium Dichromate.*—A tenth-normal solution, made by dissolving exactly the calculated weight of a salt of known purity, or standardize as directed on page 74. Five hundred cubic centimeters of this solution will be sufficient.

(*b*) *Potassium Iodide.*—Prepare 200 cc of a solution containing approximately 30 gm of the solid.

(*c*) *Starch.*—Moisten 1 gm of potato starch with enough cold water to make a thick paste. Heat 100 cc of water to boiling and pour it into the starch paste. Boil gently, with constant stirring, for about a minute. The solution does not keep well and it should be made each day, as required. The addition of preservatives, such as chloroform or zinc chloride, has been tried but the solution deteriorates, even with such additions.

(*d*) *Sodium Thiosulphate.*—Prepare an approximately tenth-normal solution, calculating the equivalent weight from the following equation:

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI.$$

In weighing the crystallized salt, calculations must include 5 molecules of water of crystallization, the formula being $Na_2S_2O_3.5H_2O$.

Standardize the thiosulphate solution as follows: Pipette 25 cc of the dichromate solution into an Erlenmeyer flask and add 10 cc of potassium iodide, 10 cc of concentrated hydrochloric acid and 100 cc of water. Iodine is liberated according to the equation:

$$K_2Cr_2O_7 + 6KI + 14HCl \rightarrow 8KCl + 2CrCl_3 + 7H_2O + 3I_2.$$

Titrate immediately with sodium thiosulphate, adding 1 cc of starch solution after most of the iodine has disappeared. If starch is added too soon a blue precipitate will be produced and the end point will be reached too early in the titration.

The solution of chromium chloride, formed by the reduction of potassium dichromate, is green. The solution has an amber tint as long as free iodine is present. Upon addition of starch the solution acquires a blue-green color and the change to emerald green at the end point may be difficult to judge at first trial. By setting aside for comparison a solution that has been over-titrated, the detection of the color change will be made easier.

(*e*) *Iodine Monobromide.*—First test the laboratory stock of glacial acetic acid to insure the absence of reducing matter. Add a drop of sulphuric acid and two or three drops of potassium dichromate solution to 10 cc of acetic acid, and warm. The yellow color should persist, without the appearance of green chromium salts.

Dissolve 13.6 gm of powdered iodine in 825 cc of glacial acetic acid. The mixing machine shown in Fig. 51, page 236, will be found useful for hastening solution. Cool, decant to insure that no particles of iodine remain undissolved, and mix. Measure from a burette 25 cc of the solution into a 250-cc Erlenmeyer flask, add 15 cc of potassium iodide solution (*b*) and 100 cc of water, and mix. Titrate at once with tenth-normal sodium thiosulphate solution.

From a small burette measure 3 cc of bromine into 200 cc of glacial acetic acid. Mix and titrate 5 cc of the solution against sodium thiosulphate solution, adding potassium iodide and water as in the iodine titration. From these titrations calculate the volume of bromine solution that would be equivalent to 800 cc of iodine solution. Add this quantity of bromine solution to the iodine in a glass stoppered bottle and mix well. This should produce a solution of iodine monobromide, containing only a very slight excess of either bromine or iodine.

The addition of potassium iodide, both before and after absorption by the oil, gives a titration which may be calculated as though iodine were the only halogen present, since this element is titrated at the end, in both cases:

$$IBr + KI \rightarrow KBr + I_2.$$

(Of course the iodine is then present as KI_3.)

Determination of Iodine Number.—Half fill a 20-cc weighing bottle with oil, place in it a piece of glass rod and weigh without the stopper. Carefully pour about 0.25 gm of the oil into a 500-cc bottle or flask having a ground glass stopper, using the glass rod to assist in the transference. Reweigh and prepare another sample in the same manner.

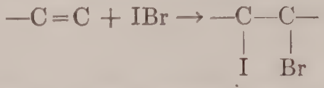
Dissolve the weighed sample of oil in 10 cc of chloroform then add 25 cc of iodine monobromide solution, measuring from a pipette. Stopper, mix and allow to stand for 30 minutes, shaking occasionally. The bottle should not be left in strong light.

At the time that the iodine monobromide solution is measured into the oil solution, measure the same amount of solution into two bottles, containing the chloroform but no oil. Treat these in exactly the same manner as the solution containing oil. This is for the "blank" determination.

At the end of the absorption period add 15 cc of potassium iodide solution (*b*). Add 100 cc of water, washing down any iodine that may be on the stopper. Titrate the unabsorbed iodine with standard sodium thiosulphate, shaking constantly. When only a faint yellow remains add 1 cc of starch solution and finish the titration. At the last the bottle should be closed and shaken until all iodine remaining in the chloroform has been extracted by the potassium iodide. The temperature should be kept as nearly constant as possible throughout the experiment.

From the volume of sodium thiosulphate required for the iodine solution alone subtract that required for the oil and iodine solutions. The remainder is the volume corresponding to the absorbed iodine. Calculate the per cent of iodine absorbed.

Iodine monobromide is absorbed at a double bond thus:



$$-\mathrm{C}=\mathrm{C} + \mathrm{IBr} \rightarrow -\underset{\mathrm{I}}{\underset{|}{\mathrm{C}}}-\underset{\mathrm{Br}}{\underset{|}{\mathrm{C}}}-$$

Acid Value.—Fresh oils sometimes contain small amounts of free fatty acids produced during the process of extraction. Rancid fats and oils contain free acids as products of hydrolysis of the glycerides composing them. The acid value is defined as the *number of milligrams of potassium hydroxide required to neutralize the free fatty acids in* 1 *gm of oil or fat.* Acidity is also sometimes expressed in terms of oleic acid as per cent, or as "acid degree," which is *cubic centimeters of normal base equivalent to the free acids in* 100 *gm of oil or fat.* The determination of acid value is made for the purpose of determining the condition of the oil and its fitness for a given use, rather than for the purpose of identifying it, since the acid value is a variable within rather wide limits for any oil.

Determination of Acid Value.—Weigh 20 gm of oil or fat into a 200-cc flask and add 50 cc of 95-per cent alcohol which has been made neutral to phenolphthalein by a dilute solution of sodium hydroxide. Heat to the boiling-point in a steam bath and agitate thoroughly. Titrate with a tenth-normal solution of sodium or potassium hydroxide, using phenolphthalein. Shake vigorously during the titration and add the standard solution until the pink color persists for a short time. An absolutely permanent color cannot be obtained because any excess of base will finally saponify the oil and thereby become neutralized.

Saponification (Köttstorfer) Number.—The saponification number is the *number of milligrams of potassium hydroxide required to saponify* 1 *gm of oil or fat.* Different oils show different saponification numbers because of variation in the molecular weight of the esters contained in them, those of relatively low average molecular weights requiring more base for the saponification of a given weight of oil than those of higher molecular weights. The variation is, however, not as great as is the case with iodine absorption numbers and the saponification number is consequently not as valuable for use in identifying oils as is the iodine number.

Notable exceptions to this rule are butter and cocoanut fat, on the one hand, and the true waxes on the other. Of these the first group contains appreciable quantities of the glycerides of butyric, caproic and caprylic acids, in addition to those of oleic, palmitic and stearic acids, which make up the bulk of most other oils and fats. The lower molecular weights of these acids raises the saponification number of butter to about 227 and that of cocoanut fat to 255.

The true waxes are not glycerides but esters of mono- and di-hydric alcohols, usually of higher molecular weights than that of glycerol and always of higher equivalent weights. Most waxes contain also acids of higher molecular weight than that of stearic acid, as constituents of the essential esters. This gives lower saponification numbers to waxes, as will be noted from an inspection of Table XII on page 198.

It will thus be seen that the determination of saponification number will be useful chiefly in identifying materials of the classes just named. In most other cases this constant will fall between the approximate limits of 190 and 210.

Insoluble Acids (Hehner Value) and Soluble Acids.—The determination of the saponification number may be conveniently combined with the determination of soluble acids and insoluble acids. Among the most important of the acids of smaller molecular weight than oleic acid, combined as glycerides, are butyric, caproic, caprylic and capric acids, discussed above. These acids are soluble in water, the solubility decreasing as the molecular weight increases, so that, while butyric acid is infinitely soluble, capric acid dissolves only to the extent of 1 part in 1000 parts of boiling water. The next acid in the series, lauric acid, is almost insoluble while the still higher acids are practically insoluble. An approximate separation of the lower acids from the higher ones may be accomplished by saponifying the oil, decomposing the resulting soap with sulphuric acid and washing the fatty acids with water. The *per cent of insoluble* acids is called the *Hehner value*.

An inspection of the formula for a typical triglyceride, as that of palmitin, $C_3H_5(C_{16}H_{31}O_2)_3$, shows that the acid residue comprises the greater part of the compound. Also since the variation in the molecular weights of the three acids, palmitic, stearic and oleic, which make the greater part of the acids of most oils and fats, is small as compared with the molecular weights themselves, it is not to be expected that there would be a large variation in either the Hehner value or the per cent of soluble acids. The former has an average value of about 95 and the latter of considerably less than 1. *Therefore these numbers are without any great significance in most cases* and their determination will give little assistance in the task of identifying most oils. A few exceptions to this statement should be noticed.

Butter has already been mentioned as containing unusually large quantities of butyric, caproic, caprylic and capric acids. Consequently its Hehner value falls to 88–90 and its per cent of soluble acids rises to about 5. Other notable exceptions are *cocoanut*, *palm nut* and *croton* oils. Practically, it is in these cases only that the determination of soluble and insoluble acids will be of any great use.

Determination of Saponification Number.—Prepare the following solutions:

(*a*) *Alcoholic Base.*—Purify 2 liters of alcohol by heating on a steam bath for 3 hours with about 10 gm of sodium hydroxide, using a reflux condenser. Distill and make 1000 cc of a solution of 40 gm of potassium hydroxide in the alcohol. The potassium hydroxide should be as nearly free from carbonate as is possible. Allow the solution to stand until the small amount of potassium carbonate that is always present has settled out, then decant into another bottle. The concentration does not remain constant for long and the solution should not be standardized, except by a blank determination, made at the time saponification number is determined.

(*b*) Prepare also a *half-normal solution of hydrochloric acid in water.*

Select two ordinary flasks of 250-cc capacity having, if possible, necks of slightly larger diameter at the top than at the bottom, though this feature is not essential. Clean with alcohol. Weigh into each flask about 5 gm of oil or fat, using a small bottle and glass rod as in the determination of iodine number. Add to each flask 50 cc of the alcoholic solution of potassium hydroxide from a calibrated pipette or burette, place in the neck of the flask a funnel having a short stem and warm on the water bath until the alcohol boils, though it should not be evaporated more than is necessary. The oil is usually saponified in about 30 minutes. A homogeneous solution must be produced, so that no separation will occur when boiling is interrupted. Measure 50 cc of the alcohol solution of potassium hydroxide into each of two other flasks, for standardization. While saponification of the oil is proceeding titrate these solutions with the half-normal acid, using phenolphthalein. Cool the flasks in which the oil was saponified, add a drop of phenolphthalein and titrate the excess of base with half-normal acid, deduct from the volume used for 50 cc of the base in the standardization and calculate the saponification number.

If it is desired to determine insoluble and soluble acids the solution which has just been used for the determination of saponification number may be used for this purpose. For detailed directions refer to other works on this subject.[1]

Reichert Number and Reichert-Meissl Number.—There is no sharp line of division between the fatty acids volatile with steam and those not volatile and it is not possible to effect more than a very approximate separation by a method of distillation unless this is continued for a very long time. On the other hand fairly constant proportions of acids may be distilled if the method is rigidly standardized. In this way figures may be obtained that have a value in identifying certain oils and fats. The determination is made chiefly in the examination of butter and its substitutes. Pure butter contains volatile acids to the extent of nearly 10 per cent of the total fatty acids.

The saturated acids to and including capric acid are the only ones of the series that may be distilled without decomposition. They are therefore known as "volatile" acids while the higher acids (above lauric) decompose when distilled and are therefore called "non-volatile." Lauric acid distills with steam but is slightly decomposed. Although the volatile acids boil at temperatures higher than 100° they can be distilled with steam.

The method proposed by Reichert and modified by Meissl has been extensively adopted. It should be understood that neither method gives the correct per cent of volatile acids but simply the proportion that will be distilled under certain stated conditions. The Reichert Number is *the number of cubic centimeters of tenth-normal base required to titrate the acids obtained from* 2.5 *gm of oil or fat by Reichert's distillation process.* The Reichert-Meissl number is the same as the Reichert number *except that* 5 *gm of oil or fat is used.* The Reichert-Meissl number is not exactly double the Reichert number.

The Reichert-Meissl number of most oils, fats and waxes is less than 1 and the determination will be of little service in identifying these oils. The following oils are exceptional in this respect.

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[1] Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes;" Assoc. Off. Agri. Chemists, "Methods of Analysis;" Mahin, "Quantitative Analysis."

TABLE IX.—REICHERT-MEISSL NUMBERS

Oil or fat	Reichert-Meissl number	Oil or fat	Reichert-Meissl number
Butter fat	28.5	Mocaya	7
Cocoanut	7	Palmnut	5
Croton	13	Porpoise	47

Butter and Substitutes.—Practically speaking, the determination of Reichert-Meissl number is a test chiefly of value in the dairy laboratory. Butter substitutes are of two general classes: (*a*) Oleomargerines, made chiefly from refined lard and "oleo oil" (the olein of beef tallow) and (*b*) preparations in which cocoanut fat is one of the essential constituents. For members of the first class the Reichert-Meissl number will be less than 1, while mixtures of the second class will show numbers ranging up to 7, according to the per cent of cocoanut fat in the preparation. The number for pure butter is about 28.5, as noted in the table above.

Applications to butter testing are noted in the chapter on Dairy Products, page 223.

Spitzer and Epple[1] have constructed the chart shown in Fig. 45 for the application of Reichert-Meissl and saponification numbers to the approximate calculations of the proportion of oleo oils, cocoanut fat and butter fat in adulterated butters and butter substitutes. While no great accuracy is claimed for this procedure, it will undoubtedly give useful information in the interpretation of analytical results.

Determination of Reichert-Meissl Number.—Prepare the following reagents:

(*a*) *Sodium hydroxide* solution in water, 50 per cent by weight.

(*b*) *Alcohol*, 95 *per cent*, redistilled from sodium or potassium hydroxide.

(*c*) *Sulphuric acid*, 1 part concentrated acid in 5 parts water.

(*d*) *Potassium hydroxide*, approximately tenth-normal; standardized against standard acid, using phenolphthalein as indicator.

If the sample is either real or imitation butter it will contain water and curd. Melt and keep at 60° until the fat has separated and, if necessary, filter the fat through a dry paper placed in a hot-water funnel (Fig. 50, page 226).

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[1] *Ind. Exp. Sta. Bull.*, **254** (1921).

Ordinary flasks of 200-cc capacity, are cleaned and dried. The oil or melted fat is dropped in from a weighed bottle until 5 gm, measured to within one drop, is obtained. The oil must not be left on the neck of the flask. Record the exact weight. Add 10 cc of alcohol and 2 cc of 50-per cent sodium hydroxide solution, connect with a reflux condenser and heat upon the steam bath until the oil is saponified. Remove the condenser and evaporate the alcohol on the steam bath. Add 135 cc of recently boiled water and warm on the water bath until solution is complete, then cool. Add two or three pieces of pumice stone or about 1 gm of crushed porcelain to prevent bumping, then add 10 cc of the diluted sulphuric acid. Again attach the reflux condenser and heat on the steam bath until the acids form a clear layer. Connect the flask with a distilling tube (Fig. 46) and a condenser and distill over a flame at such a rate that 110 cc shall be obtained in approximately 30 minutes. The distillate is received in a flask which is graduated to contain 110 cc. Mix the distillate, and filter through a dry filter to remove traces of insoluble

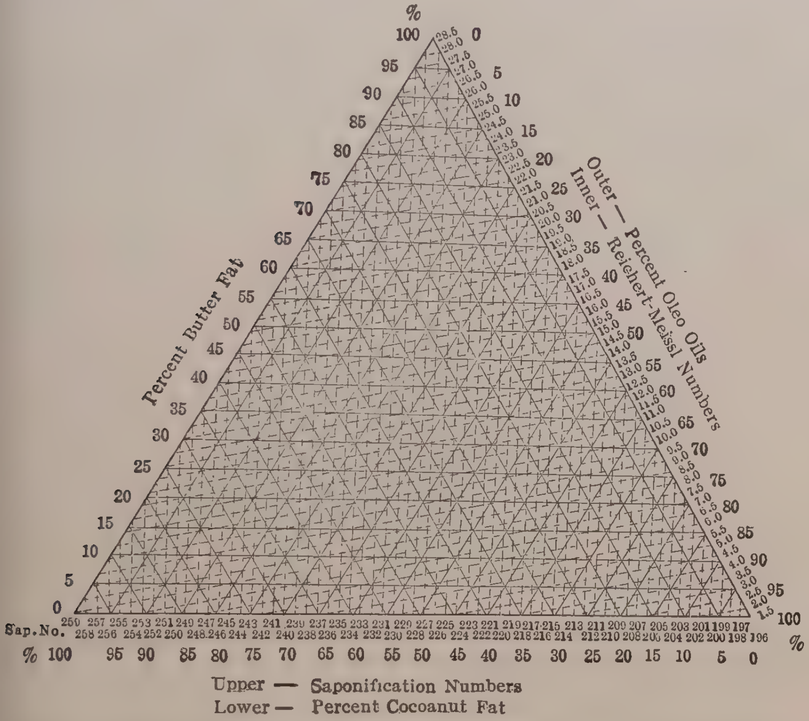

FIG. 45.—Spitzer and Epple's chart for composition of butter substitutes.

acids carried over by the steam, receiving the filtrate in a flask graduated to contain 100 cc. Titrate 100 cc of the filtrate with standard potassium hydroxide. Make the proper correction for the fact that only 100 cc of the distillate was used, also correct the number of cubic centimeters of standard potassium hydroxide used, in case this solution was not exactly tenth-normal or in case the sample weight was not exactly 5 gm. The result is the Reichert-Meissl number.

Polenske Value.—One of the very important constituents of some butter substitutes is cocoanut oil, a pure white vegetable fat having a pleasant taste and a consistency which is about the same as that of butter. Its Reichert-Meissl number is lower than that of butter, as is shown in Table IX, page 184. The volatile acids obtained from cocoanut oil in the Reichert-Meissl distillation contain much larger quantities of acids insoluble at 15° than do the volatile acids from butter. Butyric acid comprises from 60 to 70 per cent of the volatile acids from butter and this acid is soluble in water in all proportions. The volatile acids from cocoanut oil contain larger quantities of caproic, caprylic, capric and lauric acids, these being almost insoluble at 15°. The Polenske value (called by its author the "new butter value") is the *number of cubic centimeters of tenth-normal base required to titrate the insoluble acids obtained in the Reichert-Meissl distillation.*

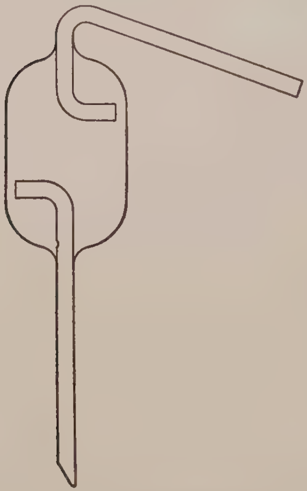

Fig. 46.—Distilling tube.

The Polenske value for pure butter varies from 1.5 to 3.0, while that for cocoanut oil varies from 16 to 18.

It is necessary to avoid the use of alcohol in the saponification of the fat and therefore the determination of Reichert-Meissl number must be modified if the two determinations are to be combined. Polenske's modification is essentially as follows:

Determination.—Saponify 5 gm of the fat by heating in a 250-cc round flask, using a reflux condenser. For the saponification use 20 gm of glycerol and 2 cc of a 50-per cent solution of sodium hydroxide in water. When saponification is complete dissolve the soap in 135 cc of recently boiled water and add 25 cc of dilute sulphuric acid (50 cc in 1000 cc of solution) and a small amount of crushed porcelain or pumice. Connect with a condenser by means of a distilling tube (Fig. 46) and distill into a flask which is graduated at 100 cc and 110 cc; the distillation should proceed at such a rate that 110 cc passes over in about 20 minutes. When the distillate reaches the 110-cc mark on the flask replace the latter by a 25-cc cylinder and stop the distillation. Immerse the flask in water at 15° and allow to remain for 15 minutes. The level of the water must be above the 110-cc mark on the flask. Mix the contents of the flask and pass through a dry, 8-cm filter and, if desired, determine the Reichert-Meissl number, using 100 cc of the filtrate. Rinse the 110-cc flask but without removing any of the insoluble acids adhering to it. Wash the filter three times with 15 cc of water, this water having previously been used for washing the condenser, cylinder and flask. Dissolve the insoluble acids from the condenser, cylinder and filter, using three successive portions of neutral (to phenolphthalein) 90-per cent alcohol and allowing the solution to run into the 110-cc flask. Titrate the alcoholic solution with tenth-normal potassium hydroxide solution, using phenolphthalein, and calculate the Polenske value.

Acetyl Value.—Compounds containing a hydroxyl group will readily combine with acetic anhydride, acetic acid and an acetyl compound being produced. This takes place with an oil containing free higher alcohols or hydroxy-acids, the latter either in the form of esters or of free acids. For example lanopalmic acid forms acetolanopalmic acid:

$$C_{15}H_{30}OHCOOH + (CH_3CO)_2O \rightarrow C_{15}H_{30}OCH_3COCOOH + CH_3COOH. \quad (1)$$

After washing out the excess of acetic anhydride the amount absorbed may be determined by saponifying the oil with an alcohol solution of potassium hydroxide, evaporating the alcohol, adding standard sulphuric or hydrochloric acid to liberate the acetic and fatty acids and either distilling the acetic acid or washing out with water, then titrating. The reactions illustrated by the case of aceto-lanopalmitin are

$$C_3H_5(OC_{15}H_{30}OCH_3COCO)_3 + 6KOH \rightarrow C_3H_5(OH)_3 + 3C_{15}H_{30}OHCOOK + 3CH_3COOK. \quad (2)$$

$$2C_{15}H_{30}OHCOOK + H_2SO_4 \rightarrow 2C_{15}H_{30}OHCOOH + K_2SO_4, \quad (3)$$

$$2CH_3COOK + H_2SO_4 \rightarrow CH_3COOH + K_2SO_4. \quad (4)$$

Effects of Soluble or Volatile Acids.—It should be noticed that whether the distillation or the filtration process is employed, the standard base required finally to titrate the acid will include that equivalent to acids other than acetic. That is, the distillation process will yield a distillate of acetic acid and volatile organic acids while the filtration process will yield a filtrate containing acetic acid and soluble organic acids. The close relation between soluble acids and volatile acids has already been discussed (page 183). To correct for the presence of these acids in the solution containing the acetic acid one may either subtract the volume of base used in the determination of soluble (or volatile) acids, or a different method may be used. As a rule this correction will be small except with oils showing a high soluble-acid number or Reichert-Meissl number and in these cases the acetyl value is nearly zero, so that it is of little use as a means for identifying the oils.

The "acetyl value" is defined to be *the number of milligrams of potassium hydroxide required to combine with the acetic acid liberated from* 1 *gm of acetylated fat or oil.* Certain oils are characterized by unusually high acetyl values and it is only in these cases that the determination will be of value for oil testing. Castor oil is the most noteworthy of these, having a value of about 150. Another class of oils having high acetyl values is composed of "blown" or "oxidized" oils. By blowing air through oils at somewhat elevated temperatures (70° to 115°) the viscosity and specific gravity are considerably increased and they become suitable for use as lubricating oils. The chemical changes that take place are not thoroughly understood but oxidation is known to occur. This is partly due to combination with unsaturated acids (evidenced by a diminished iodine absorption number) and partly to the formation of hydroxyl radicals from hydrogen. The latter change results in an increased acetyl value and this may even reach a number as great as that for castor oil.

The large variation in acetyl values recorded in Table X will indicate the value of this determination for the identification of certain oils and fats. In other cases the determination will have little value.

TABLE X.—ACETYL VALUES OF OILS

Oil or fat	Acetyl value (average)	Oil or fat	Acetyl value (average)
Castor	150	Fish	41
Colza	17	Olive	13
Cotton seed	13	Shark liver	18
Croton	20		

Abnormal Variation in Acetyl Values.—Certain abnormalities in acetyl values should be noticed and due allowance made in specific cases.

Since acetic anhydride is absorbed by the hydroxyl radical it might be expected that free acids, free alcohols or partly hydrolyzed glycerides or other esters would show such absorption and that their occurrence in oils or fats would cause these to exhibit unusually high acetyl values. This is found to be the case and, since the three classes of substances named above are the direct products of hydrolysis, it follows that rancid oils or fats will not give normal acetyl values. For example, hydrolysis of stearin will yield free stearic acid, together with distearin, monostearin or glycerol, according to the degree of hydrolysis:

$$\underset{\text{Stearin}}{C_3H_5(OC_{18}H_{35}O)_3} + H_2O \rightarrow \underset{\text{Distearin}}{C_3H_5OH(OC_{18}H_{35}O)_2} + \underset{\text{Stearic acid}}{C_{18}H_{36}O_2}, \quad (1)$$

$$C_3H_5OH(OC_{18}H_{35}O)_2 + H_2O \rightarrow \underset{\text{Monostearin}}{C_3H_5(OH)_2OC_{18}H_{35}O} + C_{18}H_{36}O_2, \quad (2)$$

$$C_3H_5(OH)_2OC_{18}H_{35}O + H_2O \rightarrow \underset{\text{Glycerol}}{C_3H_5(OH)_3} + C_{18}H_{36}O_2. \quad (3)$$

Each of these reactions produces a hydroxylated compound, which is capable of combining with acetic anhydride. The calculated acetyl values of these substances are as follows:

TABLE XI.—ACETYL VALUES OF HYDROLYZED GLYCERIDES

Compound	Acetyl value
Distearin	84.2
Monostearin	253.9
Glycerol	772.0

Glycerol and acetoglycerol are easily soluble in water and they would therefore be removed in the process of washing the acetylated oil, so that no error would result from this source. On the other hand both distearin and monostearin, as well as their acetylated products, are insoluble in water. On this account the acetyl value of the partly hydrolyzed oil would be materially increased.

The free acids produced by hydrolysis, themselves containing a hydroxyl group, will combine with acetic anhydride to a varying degree and this will still further increase the acetyl value of such rancid materials.

It is to be noted also that many of the waxes contain certain quantities of free higher alcohols and free acids. In consequence, these waxes will show moderately high acetyl values, as will be noted in table XII, page 198.

It will be obvious from these considerations that acetyl values cannot be used with safety for identifying oils unless these are reasonably fresh. This will be indicated by the acid value, which should be low.

The most important application of this determination is in the identification of castor oil. This oil is nearly pure *ricinolein*, a glyceride of ricinoleic acid. The latter is hydroxylated oleic acid,

$$CH_3(CH_2)_5CH{\cdot}OH{\cdot}CH_2{\cdot}CH=CH(CH_2)_7COOH,$$

and the glyceride, ricinolein, has a theoretical acetyl value of 159.1. Its abundance in castor oil gives the latter an actual acetyl value of about 150, a value which is far above that of any other natural oil, only blown oils approaching it in this respect.

Lastly may be mentioned the occurrence of certain quantities of free alcohols, especially in the waxes which have, on this account, appreciable acetyl values. Cholesterol, $C_{27}H_{46}OH$, in fats, oils and waxes of animal origin, and its isomers, the phytosterols, in vegetable oils, are the most important of such alcohols.

Determination of Acetyl Value.—Place about 20 gm, approximately weighed, of oil or fat in a 100-cc flask, add an equal volume of acetic anhydride, insert a short-stemmed funnel and boil gently for two hours. Cool and pour into 500 cc of water contained in a beaker. Pass a current of carbon dioxide into the beaker through a fine orifice of a glass tube to agitate the liquid and hasten the washing. Boil for 30 minutes. At the end of this time siphon out the water layer and repeat the treatment with water and boiling until the water is no longer acid, as shown by a litmus test. Separate the acetylated oil in a separatory funnel, filter in a drying oven or hot-water funnel (Fig. 50, page 226) and dry.

Weigh accurately 2 to 4 gm of the acetylated oil into a flask and saponify according to the method used in determining the saponification number, measuring the alcohol solution of potassium hydroxide accurately and running blank determinations for standardization. Evaporate the alcohol and dissolve the soap in water. Add standard hydrochloric acid in a quantity exactly equivalent to the potassium hydroxide added, warm to melt the fatty acids and filter through a wet paper. Wash with boiling water until the washings are no longer acid, testing with litmus paper by barely touching a corner to the bottom of the funnel. The combined filtrate and washings are titrated with tenth-normal base. Calculate the acetyl value according to the definition of this number.

Maumené Number and Specific Temperature Reaction.—All oils and fats react with concentrated sulphuric acid, heat being evolved. The reactions are complex and cannot be expressed by a simple equation but oxidation occurs to a considerable degree. The heat evolution varies with different oils and it is, to some extent, characteristic. The Maumené number is *the number of Centigrade degrees rise in temperature caused by mixing* 10 *cc of concentrated sulphuric acid with* 50 *gm of oil.*

A small variation in the proportion of water in the acid causes a considerable variation in the heat evolved and to this extent the figures recorded by different investigators are not comparable because "concentrated sulphuric acid," as obtained commercially, is not a substance with any definite per cent of water.

In order partly to eliminate the errors due to variation in water another determination may be made, using the same amount of acid but substituting 50 gm of water for the oil. The ratio

$$\frac{\text{Rise in temperature with oil}}{\text{Rise in temperature with water}}$$

is known as the "specific temperature reaction." This number is not subject to as great variation as is the Maumené number.

These determinations are necessarily very crude and a considerable variation may be expected, even under the best of conditions. Variable radiation is one of the important sources of error. These "constants" will be of use chiefly in the detection of drying oils, all of which show high values.

Determination of Maumené Number.—Place a beaker, about 5 by 1.5 inches, inside a somewhat larger one and pack the open space between with wool, asbestos or cotton. Cover the beakers with a piece of cardboard through which passes a thermometer. Weigh into the inner beaker 50 gm of oil. Bring concentrated sulphuric acid to the same temperature as that of the oil, and then add, under a hood, 10 cc of this acid, stirring thoroughly with the thermometer. When the acid is all in, place the thermometer in the center of the oil-acid mixture and note the highest point attained by the mercury. The total rise in temperature is the Maumené number.

Determine also the specific temperature reaction as follows: Clean the inner beaker and introduce 50 cc of water. Add 10 cc of acid as before and note the rise in temperature. The Maumené number divided by this rise is the specific temperature reaction.

The drying oils often develop so much heat that active foaming results. Such oils should be first diluted with petroleum oils or olive oil and the proper correction made in the temperature rise.

Qualitative Reactions.—If simple and reliable qualitative tests were known for all of the oils, it is not likely that the work outlined in the preceding pages would often be carried out. It has already been explained that comparatively few such tests are known because of the similarity in the composition of the various animal and vegetable oils. Aside from the mere variation in the proportion of the various glycerides, free alcohols and free acids, there are certain constituents of certain oils that will give color reactions which are characteristic. A few of those that are reliable will be described. In most cases these tests should accompany the determination of the analytical constants, rather than be substituted for them.

Resin Oil.—Polarize the oil in a 200-mm tube. If the oil is too dark in color for this purpose it may be diluted with petroleum ether and the proper correction made in the reading. Resin oil has a polarization in a 200-mm tube of from +30° to 40° on the International sugar scale (see page 130) while other oils read between +1° and −1°

Cottonseed Oil: *Halphen Test.*—Mix carbon disulphide containing about 1 per cent of sulphur in solution, with an equal volume of amyl alcohol. Mix equal volumes of this reagent and the oil in a test tube and heat in a bath of boiling, saturated solution of sodium chloride for about an hour. In the presence of as little as 1 per cent of cottonseed oil a characteristic red color is produced. Lard and lard oil from animals fed on cottonseed meal will give a faint reaction for cottonseed oil. The unknown constituent which gives the color apparently is assimilated by the animal without change.

A negative result does not prove the absence of cottonseed oil because heating the oil for 10 minutes at 250° renders it incapable of giving the color.

Sesame Oil: *Baudouin Test.*—Dissolve 0.1 gm of finely powdered sugar in 10 cc of hydrochloric acid (specific gravity 1.20), add 20 cc of the oil to be tested, shake thoroughly for a minute, and allow to stand. The aqueous solution separates almost at once. In the presence of even a very small admixture of sesame oil this is colored crimson. Some olive oils give a slight pink coloration with this reagent, but they are not hard to distinguish if comparative tests with sesame oil are made.

Arachis (Peanut) Oil.—The constants of arachis oil are almost identical with those of olive oil and the difficulties involved in detecting admixtures of the two are correspondingly great. The Renard test for arachis oil is based upon the isolation and weighing of the small amount (about 5 per cent) of arachidic acid ($C_{20}H_{40}O_2$) that occurs as its glyceride in arachis oil. The method must be carried out with great care or stearic acid ($C_{18}H_{36}O_2$), whose solubility is not far from that of arachidic acid, will be obtained and mistaken for the latter. The Renard method is fully described elsewhere.[1]

Soybean Oil.—This oil is increasing very much in importance as a commercial product, on account of the large increase in production of soybeans for food products and for feeding to farm animals. The oil possesses drying properties, having an iodine absorption number of about 136, which is not far from that of linseed oil. For this reason soybean oil is used to some extent as an adulterant of linseed and china-wood oils. It is used also very largely in the manufacture of butter substitutes and of high-grade soaps.

A modification of Settini's test[2] has been given by Newhall.[3] This is performed as follows:

> Add 5 cc of chloroform to 5 cc of the oil in a test-tube, then add a few drops of a solution of gum Arabic and 5 cc of a 2-per cent solution of uranium nitrate or acetate. Shake vigorously to form an emulsion. Soybean oil will give a characteristic lemon-yellow emulsion, while other oils will give only faint yellow or brown.

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[1] Assoc. Off. Agr. Chemists, "Methods of Analysis," 253; Mahin, "Quantitative Analysis," 2nd Ed., 383.

[2] *Chem. Abstr.*, **7**, 908 (1913).

[3] *J. Ind. Eng. Chem.*, **12**, 1174 (1920).

Newhall states that as little as 5 per cent of soybean oil may be detected in a mixture, by this test. To the limited extent to which this test has been used by the authors it has been found to be reliable but see also a criticism by Bonney and Whitescarver.[1]

Fish and Marine Animal Oils in Mixtures with Vegetable Oils.—Practically all of these oils have very considerable "drying" properties, as shown by their iodine absorption numbers. They are characterized by the presence of glycerides containing highly unsaturated acids. The peculiar "fishy" odor of these oils is probably due to the presence of the glycerides of such acids.

Absorption of bromine by unsaturated acids or their glycerides produces bromides of limited solubility and high melting point. Octobromstearin, obtained from such acids, melts at a higher temperature (above 200°) and has a lower solubility than hexabromstearin, obtained by brominating linolenin, and this also differs in a similar manner from tetrabromstearin, obtained from linolin. Therefore the separation of octobromstearin from brominated fish and blubber oils provides a means for detecting marine animal oils in the presence of vegetable oils. The test is performed as follows:

Dissolve in a test-tube about 6 gm of the oil in 12 cc of a mixture of equal parts of chloroform and glacial acetic acid. Add bromine, drop by drop, until a slight excess is indicated by the color, keeping the solution at about 20°. Allow to stand for 15 minutes or more and then place the test-tube in boiling water. If only vegetable oils are present the solution will become perfectly clear, while fish oils will remain cloudy or contain a precipitate of insoluble bromides.

Color Reactions.—A large number of qualitative tests, based upon certain color reactions, have been proposed and considerably used in the past for the detection of various oils. Color reactions produced by adding concentrated nitric or sulphuric acids may be mentioned. Almost without exception these have been found to be unreliable and they will not be described here in detail.

Hardened Oils.—Under any circumstances the analytical investigation of oils and fats offers difficulties that are often serious. The problems of the analyst are now increased many fold by the large development of the industry of hydrogenation of liquid oils.

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[1] *J. Ind. Eng. Chem.*, **13**, 574 (1921).

It has been seen that the most important difference between oils and fats lies in the larger proportion of olein in the former and of stearin and palmitin in the latter. Olein differs from stearin only in that it contains one unsaturated double bond in each oleic acid residue; the problem of saturating this group by the insertion of hydrogen, thus forming stearin, is one that has occupied the attention of chemists for many years. At the present time the hydrogenation of the cheaper liquid oils (*e.g.*, cottonseed, corn and peanut) to form edible fats is an industry that has attained large proportions. While this process changes liquid oils to solid fats, it will also make a corresponding change in any analytical constants or tests that depend upon the degree of unsaturation, as well as in the physical properties of the oil. Linolin, linolenin and glycerides of still less saturated acids will be changed to stearin. Consequently the halogen absorption number, drying properties, specific gravity, refractive index and temperature reactions will be materially altered, as will also the odor and the general appearance and consistency. It has been stated that fish oils probably owe their characteristic odor to glycerides containing highly unsaturated acids, while the somewhat similar odor of linseed oil is due to glycerides of linolic and linolenic acids. It is interesting to note that these odors are entirely lost through hydrogenation and that the fats so produced are no longer recognizable by tests depending upon unsaturation. Many special tests for other oils, such as the Halphen reaction for cottonseed oil and the Renard test for arachis oil, fail in the hydrogenated product.

From one standpoint it might appear that the determination of what oils originally formed the raw materials for the "hardened" product is not a necessary one for the analyst to solve, since the properties of the finished product are, after all, the ones that have the chief practical interest for us. Yet it may sometimes happen that the identity of the original oil or the proof that a hydrogenating process has been employed may have a legal or other significance; the development of a series of suitable tests is therefore very desirable.

TABLE XII.—CONSTANTS FOR COMMON OILS, FATS AND WAXES

Vegetable drying oils:	Specific gravity at 20°	Index of refraction at 20°	Iodine number	Saponification number	Reichert-Meissl number	Polenske value	Acetyl value	Maumené number
Candle nut	0.922	1.477	**164**	193	....	....	...	...
Hemp seed	0.924	1.477	**148**	192	....	....	...	**97**
Linseed	0.929	1.480	**180**	192	....	....	...	**127**
Poppy seed	0.922	1.475	**136**	194	....	....	...	**88**
Soybean	0.927	1.477	**136**	192	....	....	...	**90**
Tung (China wood)	**0.938**	1.520	**165**	193	....	....	...	...
Walnut	0.922	1.478	**145**	195	....	....	...	**102**
Vegetable semi-drying oils:								
Cotton seed	0.920	1.472	**110**	194	..	..	13	80
Croton	**0.947**	1.478	**105**	**212**	**13**	**26**	..	..
Maize	0.922	1.474	**119**	192	..	..	..	82
Sesame	0.920	1.474	**105**	192	..	..	..	65
Vegetable non-drying oils:								
Almond	0.915	**1.470**	**97**	192	....	....	...	52
Arachis (peanut)	0.915	**1.470**	**90**	193	....	....	...	46
Castor	**0.961**	1.478	**86**	184	....	....	**150**	46
Olive	0.914	**1.470**	**83**	190	....	....	13	46
Rape	**0.912**	1.472	**98**	**175**	....	....	15	60
Marine animal oils:								
Codliver	0.922	1.480	**168**	**182**	....	....	..	**115**
Menhaden	0.927	1.480	**156**	192	....	....	**40**	**126**
Seal	0.925	1.475	**145**	192	....	....	16	**92**
Whale	0.922	1.476	**125**	190	....	....	..	**92**

TABLE XII—(*Continued*)

Land animal oils:	Specific gravity at 20°	Index of refraction at 20°	Iodine number	Saponification number	Reichert-Meissl number	Polenske value	Acetyl value	Maumené number
Neatsfoot Sheep's foot	**0.912**	**1.467**	**70**	195	....	....	22	50
Vegetable fats:	at 60°	at 60°						Melting point, °C.
Cacao butter	0.929	1.450	**36**	194	....	....	...	33
Cocoanut oil*	**0.897**	1.441	**9**	**255**	**7**	**17**	...	**25**
Japan wax*	**0.744**	1.450	**10**	**225**	....	....	28	52
Myrtle wax*	**0.964**	1.444	**3**	209	....	....	...	40
Palm oil*	**0.890**	1.450	**54**	200	....	....	18	40
Animal fats:								
Butter	**0.902**	1.448	**35**	**227**	**28.5**	**2.3**	...	31
Lard	**0.905**	1.454	**60**	196	....	....	...	42
Lard oil	.....	......	..	...	....	....	...	..
Tallow (beef and mutton)	0.915	1.451	**42**	195	....	....	...	48
Waxes:								
Beeswax	0.938	1.450	**9**	**94**	....	....	15	**63**
Carnauba wax	**0.964**	**1.463**	**13**	**85**	....	....	**55**	**84**
Spermaceti	0.901	1.440	**4**	**127**	....	....	...	50
Sperm oil*	(at 20°) **0.851**	(at 20°) **1.468**	**86**	**130**	....	....	...	..
Wool wax	0.913	1.473	**23**	**100**	....	....	23	35

* Note: Terms noted are misnomers. Cocoanut "oil," Japan "wax," myrtle "wax" and palm "oil" are vegetable fats, not oils or waxes. Sperm "oil" is a liquid wax.

Analytical chemistry has made little progress in this direction. The application of delicate tests for metals (nickel, palladium, etc.) that are used as catalyzers in the hardening process, may sometimes serve to prove that the material is a hardened oil, rather than a natural fat. Other than this one can say very little. But the knowledge of the nature of the changes caused by hydrogenation should serve to make the analyst more cautious than he might otherwise be when interpreting the results of his analytical data on oils or fats of unknown origin.

Interpretation of Analytical Data.—In the discussion of each so-called "constant," in the foregoing pages it has been shown that each determination will be of importance in the identification of a certain limited number of oils, fats or waxes and that in cases other than these the figures will give only negative results. The materials for which such figures have proved to be of significance were given in most of the discussions of these determinations.

In Table XII the various "constants" for a number of the more common oils, fats and waxes are collated and the ones that are of particular value in each case are printed in bold face type. The iodine number will be of value in practically all cases, since it is characteristic of classes, even when not of the individuals of a given class.

Although only one value is given in each case, it should be remembered that these are merely approximate averages and exact agreement with experimental results should not be expected. Where blanks occur in the table this is either because no value is on record or because the figure is so low as to be practically negligible.

For additional special tests and for a complete description of the individual oils, consult special treatises on the subject, such as Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," and Fryer and Weston, "Oils, Fats and Waxes."

CHAPTER XI

DAIRY PRODUCTS

The rapid development of the dairy industries in recent years has made it imperative that dairy products be standardized to a greater degree than ever before. In most of the states legal standards have been established for dairy products so that it is unlawful to offer such food materials for sale unless they conform to certain rigid requirements as to composition and cleanliness. The standardizing of dairy products has thus made necessary the services of many technically trained men, not formerly required.

MILK

The milk of different mammals varies greatly in composition, depending to a great extent upon the time required for their young to reach maturity. This is shown in the following table:

TABLE XIII.—AVERAGE PERCENTAGE COMPOSITION OF MILK OF VARIOUS KINDS

Kind of milk	Water	Total solids	Protein		Fat	Lactose	Ash
			Casein	Albumin			
Human..............	87.6	12	0.8	1.2	3.7	6.4	0.3
Cow................	87.3	12	2.9	0.5	3.7	4.9	0.7
Goat...............	86.9	13	2.9	0.9	4.0	4.6	0.8
Sheep..............	83.6	16	4.2	1.0	6.2	4.7	0.9
Buffalo (Indian)......	82.2	..	4.3	0.4	7.5	4.7	0.8
Camel..............	87.1	..	3.5	0.4	2.9	5.4	0.7
Reindeer............	67.2	..	8.4	1.5	17.0	2.8	1.4
Horse..............	90.6	9	1.3	0.7	1.1	5.8	0.3
Swine..............	84.0	..	7.23		4.5	3.1	1.0
Whale..............	48.6	..	7.11		43.6	...	0.4

It is thus seen that the milk of most mammals has been analyzed and its composition determined but, for practical purposes, the analyst rarely has to do with any other than cow's milk and human milk. The analysis of cow's milk may be made for purely scientific purposes as, for instance, the determination of the relation between the composition of milk and the breed of animal, the season of the year or the rations upon which the animal is fed, or the determination of the changes that occur in composition during the period of storage, and other similar questions. The analysis may also be made for purposes of legal control to detect sophistication. The analysis of woman's milk is usually made for hygienic purposes, in order to provide a basis for modification of the mother's diet, in cases where the infant is not thriving.

The percentage composition of milk varies rather widely although the same substances are found in practically all milk from a given species of animal. It is therefore not possible to fix, by legal enactment, the exact composition of milk that is to become an article of commerce, but certain minimum figures are usually established by law and any milk containing a constituent in quantity below the legal minimum is considered to be adulterated.

Milk is a very complex fluid, secreted through the alveoli cells of the udder. The fat is present as a suspension (emulsion) of very small globules. The milk sugar and inorganic salts are present in true solution while the proteins, casein, albumin, globulin and fibrin, are in colloidal solution. According to Babcock, the composition of cow's milk is as follows:

Milk 100.0	Fat..... 3.6	Olein		Glycerides of insoluble and non-volatile acids	3.3	Fat.......	3.6
		Palmitin					
		Stearin					
		Myristin					
		Butin (trace)					
		Butyrin		Glycerides of soluble and volatile acids	0.3		
		Caproin					
		Caprylin (trace)					
		Caprin (trace)					
	Milk serum.. 96.4	Casein.........	3.0	Containing nitrogen proteids	3.8	Solids not fat.......	9.1
		Albumin.......	0.6				
		Lactoglobulin	0.2				
		Galactin					
		Fibrin (trace)					
		Lactose..............................			4.5		
		Citric acid..........................			0.1		
		Potassium oxide.......	0.175	Ash...	0.7		
		Sodium oxide..........	0.070				
		Calcium oxide.........	0.140				
		Magnesium oxide......	0.017				
		Ferric oxide...........	0.001				
		Sulphur trioxide.......	0.027				
		Phosphorus pentoxide..	0.170				
		Chlorine..............	0.100				
		Water..					87.3
							100.0

Preparation of Sample.—When milk is allowed to stand the fat rises slowly to the top. Before the analysis is started it is therefore necessary to mix the milk thoroughly by pouring from one vessel to another several times, but without shaking vigorously as air would thus be incorporated with the liquid anid there would be also a coalescence of fat globules.

The following table will serve to indicate how far each determination must be carried before it can be stopped with safety:

TABLE XIV.—PROGRESS OF DETERMINATIONS

Determination	Stage after which work may be interrupted
Specific gravity........	Cannot be delayed
Acidity................	Cannot be delayed
Total solids............	Evaporation of sample
Ash....................	Weighing sample
Total nitrogen.........	Addition of sulphuric acid
Casein and albumin....	Filtration of proteins and addition of sulphuric acid
Lactose................	Addition of mercuric iodide or nitrate solution
Fat, paper coil.........	Drying milk on paper coil
Röse-Gottlieb......	Extraction of the fat
Babcock..........	Addition of sulphuric acid

It may sometimes be impossible to begin the analysis before bacterial action begins. In such a case add formaldehyde at the rate of 1 cc of the 40-per cent solution to 2 liters of milk.

Specific Gravity.—This determination is usually made with a lactometer, which is a hydrometer of a special form. However, it can be determined also by a Westphal balance or a picnometer. For a discussion of the use of these instruments, see pages 96 to 100, Part II.

Added Water.—As a means for detecting adulteration the specific gravity determination alone is of little value. The specific gravity of butter fat is about 0.93 and of milk solids other than fat is 1.5, while that of whole milk is 1.030 to 1.034. If water is added the specific gravity is lowered but if milk is skimmed the specific gravity is raised because the lighter portion has been removed. Therefore fat could be removed and water added in such a way as to keep the specific gravity unchanged.

A more certain method for the detection of added water is in the examination of milk serum, from which all of the fat and proteins have been removed.

Determination of Specific Gravity.—A sample of fresh milk is thoroughly mixed by pouring from one vessel to another several times, avoiding violent agitation. Determine the specific gravity at 20° within 2 minutes after mixing.

Detection of Added Water.—The ash and milk sugar are the least variable constituents of milk and they afford a suitable basis for the detection of adulteration. A clear serum may be obtained by precipitating the proteins with acetic acid or copper sulphate or by spontaneous souring, and filtering. Examine the filtrate for ash, also for other dissolved solids by means of a dipping refractometer. This instrument is described on page 118, Part II.

Examination of Acetic Serum: (*a*) *Zeiss Dipping* (*Immersion*) *Refractometer Reading.*—To 100 cc of milk at a temperature of 20° add 2 cc of 25-per cent acetic acid (specific gravity 1.035) in a beaker and heat the mixture, covered by a watch glass, by immersing in a water bath at 70° for 20 minutes. Place the beaker in ice water for 10 minutes and separate the curd by filtering through a 12.5-cm folded filter. Transfer about 35 cc of the serum to one of the beakers that accompanies the temperature control bath used in connection with the Zeiss dipping refractometer or fill the metal cup that is attachable to the instrument; take the refractometer reading at 20°, using a thermometer graduated to tenths of degrees. A reading below 39 indicates added water. If the reading is between 39 and 40 the addition of water is not certain but is to be suspected.

(*b*) *Ash.*—Transfer 25 cc of the serum to a flat bottomed platinum dish and evaporate to dryness on a steam bath, then heat over a low flame until the solids are thoroughly charred. Place the dish in a muffle furnace and ignite to a white ash at a temperature not higher than 500°, cool and weigh. Express the results as grams per 100 cc. Multiply by the factor 1.02 to correct for the dilution by addition of acetic acid. The result is the ash on the undiluted sour serum. An ash content below 0.715 gm per 100 cc indicates added water.

Examination of Sour Serum: (*a*) *Zeiss Dipping Refractometer Reading.*—Allow the milk to sour spontaneously, filter and determine the dipping refractometer reading of the clear serum at 20°. A reading below 38.3 indicates added water.

(*b*) *Ash.*—Determine the ash in 25 cc of the sour serum, using the method as directed for ash of acetic serum. Ash lower than 0.730 gm per 100 cc indicates added water.

Examination of Copper Serum: *Zeiss Dipping Refractometer Reading.*—Use a solution of copper sulphate containing 72.5 gm per liter, adjusted if necessary to read 36 at 20° on the scale of the dipping refractometer. To one volume of this solution add four volumes of milk. Shake well and filter. Determine the refractometer reading of the clear serum at 20°. A reading below 36 indicates added water.

Acidity of Milk.—Acidity of milk is due to acid phosphates and lactic acid, the latter being produced by bacterial action upon milk sugar. This is the "souring" of milk.

Determination of Acidity.—Place 20 cc of milk of known specific gravity in a 100-cc porcelain casserole and add tenth-normal (to phenolphthalein) sodium hydroxide from a burette, using phenolphthalein as an indicator, until a pink color appears and remains for 1 minute. Calculate the per cent of lactic acid, $HC_3H_5O_3$, in the milk.

Total Solids.—In order to dry the solids rapidly without decomposing them it is desirable to use a weighed flat porcelain or aluminium dish in which has been placed enough sand or asbestos fiber to cover the bottom. The sand or asbestos increases the drying surface and hinders the formation of a scum, which would interfere with the evaporation of the liquid beneath. The solid thus formed should be nearly white except as it may be colored by sand. If there is any considerable browning or blackening it is probable that the milk sugar has been partly caramelized and the resulting loss would therefore not indicate correctly the evaporated water.

Determination of Total Solids.—Use a flat porcelain or aluminium dish, 6 to 10 cm in diameter, and add 10 to 15 gm of white sand. Heat the dish and sand to constant weight at 100°, then add about 5 gm of milk, cover and reweigh; or add 5 cc of milk to the weighed dish and sand and calculate the weight from the specific gravity. Dry at 100° until the weight is constant. Cool in a desiccator and weigh rapidly. Calculate the per cent of solids.

Ash.—The ash does not represent all of the inorganic constituents of milk in their original combinations because certain changes take place during the burning of the organic matter. The ash should not be heated to a temperature higher than 600° as the chlorides of sodium and potassium might be volatilized at a higher temperature. Nitric acid may be added to aid in oxidizing the organic matter.

Determination of Ash.—Weigh accurately a flat platinum or porcelain dish holding 25 to 30 cc. Add 20 cc of milk of known specific gravity or obtain the weight by direct weighing. In the latter case the dish must be covered before and after adding the milk. Add five drops of concentrated nitric acid and evaporate to dryness on the steam bath, then ignite at a temperature just below redness until white. Cool in a desiccator and weigh. Calculate the per cent of ash.

Fat.—The fat contained in milk is usually given more consideration than any other constituent, since milk is bought and sold largely on the basis of its fat content. To some extent this is unfortunate as it has tended to underrate the other constituents, which may be of equal or greater value as food.

"Paper Coil" Method.—In this method the milk is absorbed on porous fat-free paper and dried. In this condition the fat is easily and quickly extracted as most of it is on the surface of the paper and it is thus somewhat separated from the proteins present. Ether is generally used to extract the fat. This must be anhydrous in order to avoid dissolving some of the milk sugar present. Petroleum ether is sometimes used but it has the disadvantage of dissolving fats more slowly than ordinary ether. The fat extractor, shown in Fig. 41, page 146, is used for the determination. If other forms of extractors employing cork stoppers are used the corks must be made free from ether-soluble waxes and resins by previous extraction with ether and they must fit tightly enough to prevent the escape of any considerable amount of ether.

Determination of Fat: *Paper Coil Method.*—Secure fat-free paper by extracting heavy filter paper with ether, or use the strips of extracted porous paper as obtained in the market for this purpose. These are usually about 6.5 cm wide and 55 cm long. Place the strip of paper on a clean surface and by means of a pipette distribute 5 cc of the milk over the paper. The weight of the sample is determined from the specific gravity and the volume. Roll the paper so that it will go into the extraction apparatus, bind with a fine wire and place on a watch glass in an oven. Dry at 100° for about 2 hours. Meanwhile the fat tube (*a*) connected with the extractor of Fig. 41 is dried and weighed. The dried paper and milk are placed in the extraction tube (*c*) and ether is added until it siphons over automatically. Heat the apparatus by steam or electricity. Continue the extraction for at least 2 hours. Finally disconnect the apparatus just before the ether is ready to siphon over and remove the extraction tube containing the paper. Evaporate the ether remaining in the lower cup (*a*) on the steam bath, finally completing the drying in the oven at 100°. Weigh the fat and cup, after cooling in a desiccator, and calculate the per cent of fat present. See that no flames are near when ether is being handled.

Röse-Gottlieb Method.—This method, with some slight modifications, is being widely used at present for the determination of fat in whole milk, skim milk, milk powders, and condensed or evaporated milk. Before fat can be separated from milk it is necessary to get the casein in solution or semi-solution. This is accomplished in this method by adding ethyl alcohol and dissolving the casein in ammonium hydroxide. The method is particularly applicable to powdered, condensed and evaporated milk because it is possible thus to extract fat that is mechanically enclosed in the dried casein.

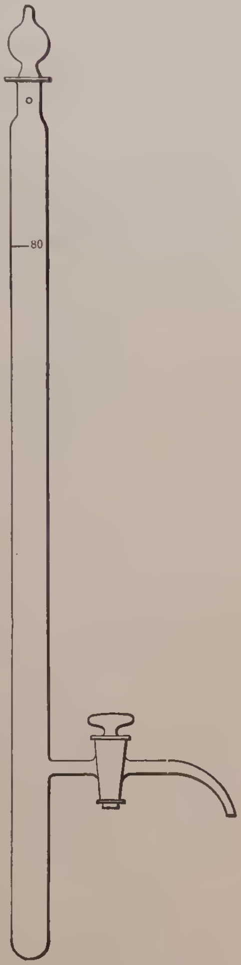

Fig. 47.—Röhrig tube.

Determination of Fat: *Röse-Gottlieb Method.*—Place 10 cc of milk in a Röhrig tube (Fig. 47) or other similar tube and add 2 cc (2.5 cc if milk is sour) of concentrated ammonium hydroxide. Mix thoroughly by inversion of the stoppered tube. Add 10 cc of 95-per cent alcohol and mix again. Add 25 cc of ethyl ether, stopper the tube and shake vigorously for 30 seconds, then add 25 cc of petroleum ether (distilled below 60°) and stopper and shake again for 30 seconds. Let stand until the bubbles of air have disappeared from the lower layer. Both layers should be clear and free from suspended particles. Draw off most of the upper layer of ether-fat solution by opening the stop cock and tipping slightly to make the separation more complete, but without removing any of the lower layer. The fat solution is run through a small (about 5 cm), dry filter, into a dried and weighed fat flask. Repeat the extraction, using 15 cc of each ether as before. Wash the tip of the outlet tube, the funnel and the filter with a small amount of the ether mixture, then evaporate the ether from the fat and ether mixture.

Dry the flask at 100°, cool and weigh. Calculate the per cent of fat in the sample.

Babcock Method.[1]—This method is rapid and convenient for general dairy control testing. The test is based upon the fact that concentrated sulphuric acid will dissolve all proteins in milk or cream and thus enable the fat to separate when whirled rapidly in a centrifuge. When the acid is added to the milk, the casein is first precipitated and then dissolved in the excess of acid. The solution darkens because of the charring of the milk sugar, due to the heat of reaction.

It is important that the acid should have a specific gravity of 1.82 to 1.83. If the acid is too dilute the fat will have a white appearance with gray particles beneath it, while if too concentrated the fat will be dark colored with black charred particles beneath. The temperature of the fat should be about 60° (140° F.) when the fat reading is made. Appreciable errors will result from volume changes if the temperature of reading is allowed to vary more than 10° (18° F.) either way. The fat should have a clear, golden yellow color and it should be separated clearly from the chocolate-colored acid solution beneath.

Standard Babcock Test Bottles.—The standard Babcock test bottles for milk and cream are as follows:

1. *Eight-per cent,* 18-*gram,* 6-*in. Milk Test Bottle.*—The total per cent graduation is 8. The total height of the bottle is 150 to 165 mm. The capacity of the bulb up to the junction with the neck is not less than 45 cc. The graduated portion of the neck has a length of not less than 63.5 mm, and the neck is

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[1] *Wis. Exp. Sta. Bull.,* **24,** (1890); *Milch-Ztg.,* **19,** 746 (1890).

cylindrical for at least 9 mm below the lowest and above the highest graduation marks. The graduations represent whole per cents, halves and tenths of a per cent.

2. *Fifty-per cent, 9-gram, 6-in. Cream Test Bottle.*—The total per cent graduation is 50. The total height of the bottle is 150 to 165 mm. The capacity of the bulb up to the junction with the neck is not less than 45 cc. The graduated portion of the neck has a length of not less than 63.5 mm and the neck is cylindrical for at least 9 mm below the lowest and above the highest graduation marks. The graduations represent five per cents, whole per cents and halves of a per cent.

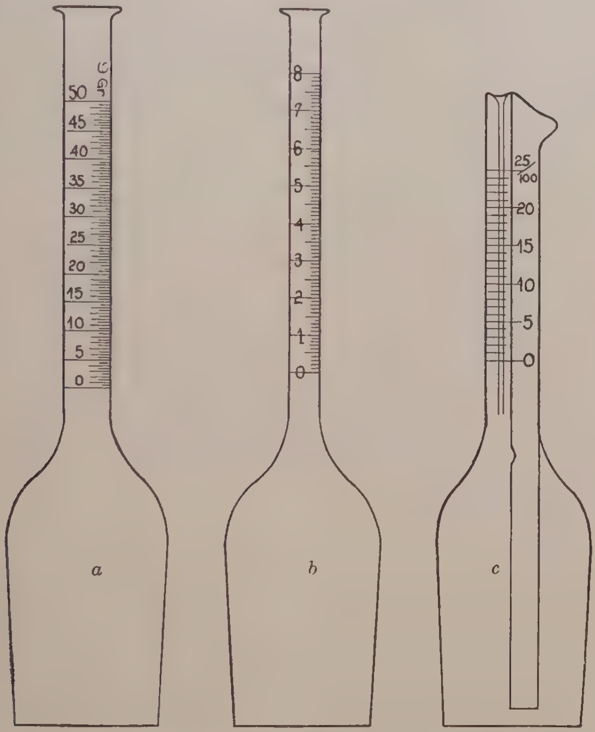

FIG. 48.—Test bottles for fat in (*a*) cream, (*b*) milk and (*c*) skim-milk.

3. *Fifty-per cent, 9-gram, 9-in. Cream Test Bottle.*—Same as (2) except that the total height of the bottle is 210 to 225 mm.

Certain forms of test bottles are illustrated in Fig. 48.

Standard Babcock Milk Pipette.—This pipette is graduated to deliver 17.6 cc of water at 20° in 5 to 8 seconds.

Calibration.—The official method for calibrating Babcock test bottles is to fill the dry bottle to the zero mark with pure mercury at 20°, weigh, fill to the highest mark and reweigh, calculating the bulb and stem capacities on the basis of 13.5471 gm of dry mercury for each cubic centimeter at 20°.

It is difficult to see what advantage this possesses over the method of calibrating by weighing water at 20° especially since the Babcock bottle filled with mercury must weigh more than 600 gm. Accurate weighing of such a quantity would require a special balance, as sensitive as the analytical balance and having large capacity.

Milk pipettes and graduates are calibrated according to the official method by measuring in a burette the quantity of water delivered by the instrument at 20°. Unless care has been exercised in wetting the inner surface of the burette, using the standard method by which the burette was calibrated, this method will be subject to considerable error since all burettes are graduated for delivery and not for capacity. A better method for calibrating pipettes is described on page 46.

Determination of Fat: *Babcock Method.*—Fill a 17.6-cc pipette to the mark with mixed milk sample and deliver to the graduated test bottle. Add to this 17.5 cc of sulphuric acid (specific gravity 1.82 to 1.83), pouring it in slowly so as to form a layer beneath the milk. Prepare an even number of bottles, up to the capacity of the centrifuge. After the acid has been added to all the bottles, mix the acid and milk by giving it a gentle rotary motion, being careful to keep the liquid from collecting on the neck of the bottle. Place the bottles in the centrifuge in such a way that they will be counterbalanced and rotate for 4 minutes at the required speed for the machine used. This is about 1,000 revolutions per minute for a wheel 10 inches in diameter or 700 for a 24-inch wheel. Add hot water to each bottle until it is filled to the neck and whirl 1 minute longer. Again add enough boiling water to bring the fat column into the graduated portion of the neck and whirl for another minute. Place the bottles in a glass vessel which is filled with water at a temperature of 57° to 60°. The water should surround the neck of the bottle to a point above the fat layer. After 1 minute measure the fat column from the top of the upper meniscus to the plane of separation between fat and aqueous solution, using a pair of dividers if considered desirable.

Proteins and Total Nitrogen.—Of the nitrogenous materials in milk, the principal protein, casein, makes up about 3 per cent of the total 3.8 per cent usually present. Globulin, albumin and fibrin comprise the other 0.8 per cent. Casein and albumin together are at least 95 per cent of the total protein content and analysis shows these two proteins to contain slightly less than 15.7 per cent nitrogen. The factor to convert the per cent of total nitrogen to protein is thus 6.38.

Either the Kjeldahl or the Gunning method may be used for the nitrogen determination. These are discussed in connection with the analysis of feeds, page 149.

Determination of Total Nitrogen.—Measure out 5 cc of well mixed milk of which the specific gravity has been determined, using a pipette and delivering into a 500-cc Kjeldahl digestion flask. Determine the per cent of nitrogen by the Kjeldahl or Gunning method, described on pages 151 and 154. Multiply the nitrogen per cent by 6.38 and report as total nitrogenous material.

Formal Titration for Total Proteins.—Proteins and their derivatives are related to certain amino acids. This is illustrated by the simple dipeptide, glycylglycine, which is derived from two molecules of the amino acid glycine, as follows:

$$\underset{\text{Glycine}}{2NH_2{\cdot}CH_2{\cdot}COOH} \rightarrow \underset{\text{Glycylglycine}}{NH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2COOH} + H_2O.$$

These substances are amphoteric, possessing basic properties on account of the amino group (NH_2) and acid properties through the carboxyl. Proteins, as well as their decomposition products, will react with formaldehyde, forming derivatives of the amino acids which have lost their basic character through substitution in the amino group:

$$\underset{\text{Glycylglycine}}{NH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}COOH} + \underset{\text{Formaldehyde}}{2HCHO} \rightarrow 2CH_2N{\cdot}CH_2{\cdot}COOH + H_2O.$$

Thus the proteins of milk are neutral to phenolphthalein but upon addition of formaldehyde they become decidedly acid.

The equations given above are presented merely to show the supposed general nature of certain changes. Calculations of the results of titrations cannot be based upon these equations because the true formulas of the proteins are not known.

Determination of Total Protein: *Formaldehyde Method.*[1]—Weigh out 20 gm of milk and place in a 200-cc beaker. Add 1 cc of phenolphthalein, then add from a burette twentieth-normal sodium hydroxide (standardized using phenolphthalein) until a distinct pink color appears. This neutralizes any lactic acid present in the milk. Next add 10 cc of formaldehyde which is neutral (a single drop of twentieth-normal base should produce a distinct pink color in 10 cc) to phenolphthalein. Stir thoroughly and add twentieth-normal sodium hydroxide until a faint pink color remains after mixing. Note the total volume of basic solution added after introducing the formaldehyde and from this calculate the per cent of total protein present. One cubic centimeter of twentieth-normal base has been found by experiment to be equivalent to 0.0864 gm of milk proteins.

Casein.—Casein exists in milk in a colloidal condition. When a dilute acid or an alum solution is added casein is flocculated. To effect complete separation the acid must be very dilute because casein and other proteins are somewhat soluble in a small excess. The separation is most nearly complete at a temperature of 40°.

Determination of Casein: *Acetic Acid Method.*—To 10 cc of milk of known specific gravity, add 90 cc of water at a temperature of 40° to 42°, stir well, add 1.5 cc of 10-per cent acetic acid and allow to stand until a flocculent precipitate settles out and a clear liquid is obtained. This should not take more than 5 minutes. Filter, wash three times with cold water and add the washings to the clear filtrate. Save the filtrate and washings for the albumin determination. Place the paper and precipitate in a Kjeldahl flask and determine nitrogen by the Kjeldahl or Gunning method as described for total nitrogen. Use the factor 6.38 to convert the per cent of nitrogen to that of casein.

Determination of Casein: *Alum Method.*—To 10 cc of milk add 50 cc of water at a temperature of 40°. Then add 2 cc of a solution of potassium alum (saturated by heating 25 gm of crystals with 100 cc of water, until dissolved, then cooling to 40°) and stir. A flocculent precipitate forms and it should settle rapidly. Let the precipitate settle for 5 minutes or more and then filter and wash with cold water. Save the filtrate and washings for the albumin determination. The nitrogen is determined in the residue by the Kjeldahl or the Gunning method and multiplied by 6.38 to obtain the equivalent of casein.

Casein by Hart's Method.[2]—The methods described above are rather long and tedious. The Hart volumetric method is based upon the fact that the amphoteric casein has properties of an acid, as explained on page 209, and combines with a base in fairly constant proportions. If an excess of base be used to dissolve the casein, the uncombined base can be determined by titration. It has been found that 1 cc of tenth-normal potassium hydroxide is equivalent to 0.108 gm of casein if phenolphthalein is used as indicator. Hence, when 10.8 gm of milk is used, each cubic centimeter of tenth-normal base required will be equivalent to 1 per cent of casein. The specific gravity of average milk being 1.032, 10.5 cc may be measured with sufficient accuracy for most work.

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[1] Henriques and Sorensen: *Z. physiol. Chem.*, **64**, 120 (1909).

[2] *Wis. Exp. Sta. Res. Bull.*, **10** (1910).

Determination of Casein: *Hart's Method.*—Place 10.5 cc of milk in a 200-cc Erlenmeyer flask containing 75 cc of distilled water which has been freed from carbon dioxide by boiling, then cooled to 20°. Add 1.5 cc of 10-per cent acetic acid, warm to about 40° and filter off the casein through a 10-cm filter. Wash the paper and precipitate thoroughly, using about 250 cc of cold water. Return the paper and contents to the Erlenmeyer flask, add 75 cc of carbon dioxide-free water and a drop of phenolphthalein. To this add 10 cc of tenth-normal potassium hydroxide. Stopper carefully and shake vigorously. When solution of the protein is complete, titrate to the disappearance of the red color, using tenth-normal acid. It is necessary to run a blank as this will usually require 0.2 cc or more of the tenth-normal potassium hydroxide. The number of cubic centimeters of base used in the titration, thus corrected, will express the per cent of casein.

Albumin.—Albumin is soluble in the milk serum and is coagulable by heating to 100°. It is necessary, however, first to neutralize part of the acetic acid, if this was added to precipitate casein.

Determination of Albumin.—Neutralize the filtrate from the casein determination (acetic acid method) with tenth-normal sodium hydroxide or potassium hydroxide, using phenolphthalein, or use the filtrate from the alum precipitation of casein without neutralization. Add 0.3 cc of 10-per cent acetic acid and heat in a boiling-water bath until the precipitate becomes settled. Filter, wash with cold water and determine the nitrogen and albumin as in the casein determination, using the same factor.

A comparison of the results obtained by various methods for determining milk proteins has been made by Spitzer,[1] who concluded that where speed and convenience are important the formal titration is to be preferred.

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[1] *Proc. Ind. Acad. Sci.*, p. 173 (1915).

Lactose.—This sugar is the only carbohydrate occurring in appreciable quantity in milk. It is not as sweet as cane sugar and it is not fermentable by yeast. It readily reduces Fehling's solution (a basic solution of copper sulphate and a tartrate), forming cuprous oxide (Cu_2O) and use is made of this fact in its quantitative determination. It is optically active and this affords another method for its quantitative determination.

It is necessary to remove the proteins and fat from milk before the lactose can be determined by Fehling's solution. This is one of the objections to this method but if the milk can thus be made reasonably free from other reducing agents the method is capable of yielding fairly accurate results.

If the cuprous oxide formed were to be weighed directly there would be an error due to inclusion of organic impurities in the precipitate. This error can be lessened by heating the cuprous oxide in a muffle furnace for 20 minutes to a dull redness. This converts the cuprous oxide to cupric oxide (CuO), which can be weighed free from organic impurities, the per cent of sugar being then calculated. A better way, however, is to dissolve the cuprous oxide from the filter with dilute nitric acid and to determine the copper electrolytically or volumetrically. All of these methods are discussed in connection with feed analysis, pages 157 and 158.

Determination of Lactose: *Reduction Method.*—Pipette 25 cc of well mixed milk into a 500-cc volumetric flask. Add 350 cc of water, 10 cc of Fehling's copper sulphate solution (page 158) and 44 cc of tenth-normal sodium hydroxide or an equivalent volume of a solution of any other normality. Colloidal cupric hydroxide flocculates, carrying down fat and proteins. Copper will be present in very slight excess. Make the volume up to 500 cc and mix thoroughly. Although the apparent volume occupied by the precipitate is considerable, its actual volume is relatively small and an approximate correction is made as noted below. Filter through a dry filter and use 50-cc portions in making the lactose determination. It is necessary to follow directions carefully as the reactions taking place are modified by variation in time of heating or in the concentrations of the solutions.

Into a 400-cc Pyrex beaker, pipette 50 cc of the clarified milk serum and 25 cc each of the two Fehling's solutions prepared as described on page 158. Heat at such a rate that the boiling begins in about 4 minutes and continues for exactly 2 minutes. Keep the beaker covered while heating. Filter off the cuprous oxide through a Gooch crucible. Wash five or six times with hot water. Dissolve in a warm mixture of 2 cc of concentrated nitric acid and 2 cc of water, wash the solution through with hot water and determine copper by any of the methods used in feed analysis, described on pages 159 to 163.

Optical Methods.—The ability of carbohydrates containing one or more asymmetric carbon atoms to rotate the plane of polarization of polarized light affords the basis for an important method for their determination. The proteins of milk also are slightly optically active, hence they must be removed before lactose can be determined.

The subject of polarimetry is discussed on pages 121 to 137, Part II. This should be reread before starting the determination of lactose. The instrument designed especially for sugar determinations is called a saccharimeter. Its graduations read directly in per cent of sugar when a definite specified weight (the "normal weight)" is contained in 100 cc and when the polarization is made in a 200-mm tube. The following determination is based upon the use of an instrument bearing the International scale. (See page 130, *et seq.*)

Protein Precipitation.—By treatment of milk with an acid solution of mercuric iodide the proteins are combined with the mercury and flocculation results. If the solution is then diluted to a definite volume without previous filtration the solids present cause a volume error unless a correction is applied. It has been found that a close approximation may be made by deducting 2.6 cc for the volume of precipitate obtained from the sample as specified below, the dilution being accomplished in a flask graduated to contain 102.6 cc. If a flask graduated in this manner is not at hand, prepare one as follows: Fill a 100-cc volumetric flask exactly to the mark with distilled water. From a burette add 2.6 cc more and mark the position of the bottom of the meniscus with a strip of label. Mark permanently, if desired, by the method described on page 45, Part I.

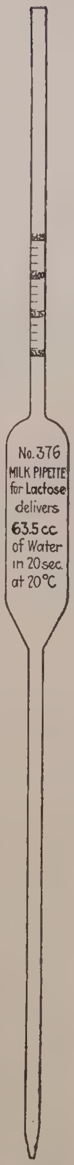

FIG. 49.—Milk pipette for lactose determination.

A still more accurate method involves the double dilution, discussed on page 133.

The normal weight for lactose, 32.90 gm (see page 131), is too small a quantity of milk for convenient accurate determinations and it is customary to use twice this amount or 65.80 gm. In order that the sample of milk may be measured instead of weighed the following table may be used and a special pipette like Fig. 49 will be found convenient.

TABLE XV.—VOLUME OF MILK FOR LACTOSE DETERMINATION

Specific gravity of milk	Volume of milk (cc) for a lactose double normal weight (International scale)
1.024	64.26
1.025	64.20
1.026	64.13
1.027	64.07
1.028	64.01
1.029	63.94
1.030	63.88
1.031	63.82
1.032	63.76
1.033	63.70
1.034	63.64
1.035	63.58
1.036	63.51

Determination of Lactose.—Prepare acid mercuric iodide solution as follows:

Dissolve 33.2 gm of potassium iodide, 13.5 gm of mercuric chloride and 20 cc of glacial acetic acid in 640 cc of water.

Determine the specific gravity of milk by means of a sensitive hydrometer or a picnometer. Refer to the table and measure, at the temperature at which the specific gravity was taken, the quantity of milk indicated in the table above, the sample having been mixed thoroughly immediately before making both measurements. The milk is run into a volumetric flask, graduated at 102.6 cc. Add 30 cc of acid mercuric iodide solution, dilute to the mark on the flask, mix well and allow the precipitate to settle. Filter through a dry filter, rejecting the first 25 cc of the filtrate and receiving the remainder in a dry flask. Polarize in a 200-mm tube, having the solution at a temperature of 20°. The reading on the sugar scale is to be divided by two because twice the normal weight of sample was taken. The quotient is the per cent of lactose in the milk.

Microscopic Examination of Milk.—There is considerable variation in the size of fat globules in the milk of different cows of the same breed or of different breeds, especially between Jersey and Holstein animals. Also, pasteurization modifies the size and grouping of fat globules. Extensive studies were made by Woll[1] on the number and size of fat globules as modified by the period of lactation, and by the age and breed of the cow. He found that the period of lactation was the most important cause of variation—that the average number of globules in 0.0001 cmm for all cows is 138, at the beginning of the lactation period, and at its end 367. The average "relative diameters" (per cent of fat divided by the number of globules in 0.0001 cmm) of fat globules was found to be 290, 217 and 177 for the Jersey, Guernsey and Shorthorn, respectively.

Determination of Number of Fat Globules.—Dilute 10 cc of a sample of fresh milk to 500 cc and mix well. Prepare six capillary tubes of about 0.1-mm inside diameter by heating 5-mm tubing and pulling it to a thread. Cut into pieces about 3 cm long. Dip the end of each capillary tube into the diluted milk (recently mixed) and when filled seal both ends of the tubes by dipping them into vaseline. Place one of the tubes on a slide upon which has been placed a drop of glycerine and determine the average inside diameter by placing it in the field of a microscope which contains a micrometer eye piece having 0.01-mm divisions. After about 20 minutes make a count of the number of fat globules contained in a section of the tube equivalent to 20 or 30 divisions of the scale. Repeat the measurements and count, using another capillary tube. From the diameter of the tube and the length of the section in which the count was made calculate the number of fat globules in 1 cubic millimeter of undiluted milk. Note the form of the fat globules in this and in a portion of the same sample pasteurized at 140° F.

In the experiment just described, the function of the glycerine is to cover the capillary tube with a fluid medium having nearly the same index of refraction as that of the glass, thus avoiding the magnifying effect of the curvature of the tube, as well as preventing the apparent distortion of form of fat globule.

Formaldehyde is very efficient as a preservative and when present in so small an amount as 1 part in 20,000, it will extend the time of milk preservation at least 24 hours at 15°. Its use in commercial milk is usually forbidden, as is that of most other preservatives.

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[1] *Wis. Exp. Sta. Ann. Rep.* **11** (1894).

Test for Formaldehyde.—In a porcelain dish mix 5 cc of milk with 10 cc of concentrated hydrochloric acid, containing 0.2 gm of ferric chloride per liter. Heat cautiously nearly to boiling and keep near the boiling point for 1 minute. A violet color indicates the presence of formaldehyde.

Test for Borates.—Make 25 cc of milk slightly basic with lime water and evaporate to dryness in a porcelain dish over the steam bath. Char the residue in the dish and when cool add 15 cc of hot water, then boil. Add hydrochloric acid drop by drop until neutral to litmus, then add an excess of about ten drops. Filter and evaporate to dryness on a steam bath. Immerse turmeric paper in the solution while the evaporation is taking place. If borax or boric acid is present, the turmeric paper will turn cherry red when dry and it will change to a bluish green when moistened with ammonium hydroxide.

Cane Sugar.—Cane sugar is occasionally present in milk which has been thickened with calcium saccharate or which has been mixed with sweetened condensed milk.

Test for Cane Sugar.—Mix 10 cc of milk in a test-tube with 0.5 gm of ammonium molybdate and 10 cc of 3-per cent hydrochloric acid. Make a blank test using milk of known purity. Place the tubes in a water bath and gradually raise the temperature to 80°. A blue color will develop in normal milk but if sucrose is present the milk remains unchanged in color. The test is quite delicate as even 1 gm in a liter may be detected by the reaction.

Heated Milk.—One method for the detection of heated milk is based upon the presence in raw milk of an enzyme which, in the presence of hydrogen peroxide, is capable of producing color changes with certain organic substances, the enzyme probably acting as a catalyzer.

Bacteria in normal fresh milk will change methylene blue to a colorless compound in about 20 minutes. But when milk has been heated these bacteria are present in greatly diminished numbers and are no longer capable of decolorizing the solution. This furnishes another test for heated milk, since by this reaction one may detect milk which has been pasteurized at 65° and held at that temperature for 30 minutes, or at 70° for 10 minutes.

Test for Heated Milk.—(*a*) To 10 cc of milk add about three drops of a freshly prepared 2-per cent solution of *p*-phenylenediamine hydrochloride and a few drops of hydrogen peroxide. Shake well. Milk which has not been heated will give a blue color.

(*b*) Prepare a test solution of methylene blue by mixing 5 cc of saturated alcoholic solution with 5 cc of 40-per cent formaldehyde and 190 cc of water. Add 1 cc of this solution to 20 cc of milk in a test-tube, and place the tube in a water bath at 45°. Cover the liquid with a layer of paraffine oil to exclude the air. Repeat the test on a heated sample of milk. It will take about 20 minutes for the unheated milk to decolorize while the heated sample will require considerably more time.

Condensed Milk.—"Condensed" milk is made by evaporating either whole or skimmed milk under reduced pressure and adding cane sugar. "Evaporated" milk does not contain cane sugar. The Federal standard provides that in evaporated milk there shall be not less than 34.3 per cent solids, including fat, that the fat content must be at least 7.8 per cent and that it must not be made up to this minimum by adding butter oil.[1] The composition of three commercial brands of evaporated milk is given in the following table:[2]

TABLE XVI.—COMPOSITION OF EVAPORATED MILK

Sample	Water	Fat	Lactose	Protein	Ash
1	70.75	9.42	9.75	8.44	1.54
2	70.90	8.35	10.37	7.86	1.62
3	72.11	8.69	9.66	7.52	1.54

The methods of analysis of condensed and evaporated milk are similar to those described for raw milk, except in the determination of cane sugar and of lactose.

Preparation of Sample: (*a*) *Unsweetened.*—Dilute 40 gm of the homogeneous sample with 60 gm of water and make the mixture uniform by pouring from one beaker to another.

(*b*) *Sweetened.*—If the can is cold, place it in water at about 35° until the temperature of the contents becomes uniform. Open, scrape out all the milk adhering to the interior of the can and mix by transferring the contents to a dish sufficiently large to permit stirring thoroughly to make the whole mass homogeneous. Weigh 100 gm into a 500-cc volumetric flask and make up to the mark with water. If the milk will not dissolve completely, weigh out each portion for analysis separately.

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[1] *Food Insp. Decision*, **131** (1911).

[2] *Ind. Exp. Sta. Bull.*, **134** (1909).

Determination of Total Solids.—Use 10 cc of the solution just prepared and dry as directed on page 204, drying on either sand or asbestos fiber.

Determination of Ash.—Evaporate 10 cc of the solution to dryness in a platinum or porcelain dish on the water bath and ignite the residue as directed on page 204.

Determination of Protein.—Determine the nitrogen in 10 cc of the solution using the Kjeldahl method as described on page 151 and multiply by 6.38 to obtain the equivalent of protein.

Determination of Fat.—Weigh 4 to 5 gm of the homogeneous sample into a Röhrig tube or similar apparatus, dilute with water to about 10.5 cc and proceed as directed on page 205.

Determination of Sucrose in Sweetened Condensed Milk.—Prepare a reagent for clarification as follows:

To 220 gm of yellow mercuric oxide add 400 cc of water and sufficient concentrated nitric acid to form a clear solution, being careful to avoid an excess. Dilute to about 900 cc and add sodium hydroxide solution, slowly and with constant shaking, until a slight permanent precipitate is obtained. Dilute to 1000 cc and filter. The solution will become acid in time, due to hydrolysis and precipitation of basic mercuric nitrate. Dilute base solution may be added to correct this.

Introduce 50 cc of the milk solution already prepared into a 100-cc volumetric flask, add 25 cc of water, mix, add 5 cc of mercuric nitrate solution and shake. Without delay, and while stirring constantly, add enough of a 2-per cent sodium hydroxide solution to render the solution neutral to litmus paper, being careful to avoid a basic reaction. Dilute to the mark on the flask, mix thoroughly and filter through a dry paper, discarding the first 10 cc of filtrate.

Polarize the filtrate in a 200-mm tube at 20°, then invert as follows: Pipette 50 cc of the filtrate into a 100-cc volumetric flask and add 5 cc of concentrated hydrochloric acid, slowly and mixing well. Set the flask aside for 24 hours at a temperature of 20° to 25°. Polarize the solution of invert sugar in a 200-mm tube at 20° and multiply the reading by two, to correct for the dilution.

Correct both direct and invert readings for the volumes occupied by the precipitated protein and fat at the time dilution was made, using the per cents of these substances as already determined and assuming a volume of 0.8 cc and 1.075 cc, for 1 gm of protein and fat, respectively. The volumes of these substances will be:

$$V = \frac{10(0.8P + 1.075F)}{100} = 0.08P + 0.1075F, \qquad (1)$$

and the corrected readings on the saccharimeter:

$$R = \left(\frac{100 - V}{100}\right)r = \left(\frac{100 - 0.08P - 0.1075F}{100}\right)r, \qquad (2)$$

where

V = volume of protein and fat precipitate,

P = per cent of protein,

F = per cent of fat,
R = corrected reading and
r = observed reading.

Ten grams is the weight of the original undiluted sample in the solution as finally used for polarization.

Calculate the per cent of sucrose by Clerget's formula, developed on page 132, Part II. Taking account of the fact that less than the normal weight of milk sample was used this formula becomes:

$$S = \frac{2{,}600(a - b)}{(142.66 - 0.5\,t)w} \text{ in which}$$

S = per cent of sucrose in the sample,
a = corrected direct polarization,
b = corrected invert polarization,
t = temperature of solution polarized (20°),
w = weight of sample taken (in this case 10 gm).

Determination of Lactose.—On account of the presence of sucrose in condensed milk, the lactose cannot be determined directly by polarization. The copper reduction method is suitable for this purpose, as sucrose does not reduce Fehling's solution.

Measure 100 cc of the milk solution already prepared into a 250-cc volumetric flask and dilute to about 200 cc. Add 6 cc of Fehling's copper sulphate solution (see page 158) and make up to the mark. Mix well, filter through a dry filter and determine lactose as directed on page 212.

Powdered Milk.—The rapid progress made in recent years in producing a high grade of powdered milk has greatly stimulated its use by bakers and confectioners. It also is used in ice cream to give body and smoothness to the product. The spray process now used in its manufacture probably owes its success to the comparatively low temperature at which evaporation and condensation take place. Milk or evaporated milk is dried by forcing a fine spray into a current of warm air, thus causing the milk particles to remain in suspension long enough to lose most of their water before depositing on the sides of the container.

For the analysis, dissolve 10 gm in water, dilute to 100 cc, mix well and proceed as outlined for condensed milk. To determine moisture, dry about 2 gm to constant weight at 100° and calculate the per cent loss.

CREAM

Commercial cream must contain not less than 18 per cent of fat according to the Federal standard. The following table gives some figures on the composition of a typical milk, cream and skim milk, the latter obtained by separating the cream with a centrifugal separator.

TABLE XVII.—COMPARATIVE COMPOSITIONS OF MILK, CREAM AND SKIM MILK

	Fat	Ash	Casein	Lactose	Total solids	Specific gravity
Milk	5.0	0.79	3.50	4.70	14.0	1.032
Cream	21.9	0.58	2.02	3.32	27.0	1.015
Skim milk	0.2	0.78	3.62	5.05	9.6	1.034

Fat.—The Babcock bottle used for testing milk is not suitable for cream on account of the higher proportion of fat. If the cream were 25 per cent fat, 18 gm would contain 4.5 gm of pure fat. Assuming 0.9 as the average specific gravity of butter fat, it will be seen that 5 cc of space would be required for the fat. Various forms of bottles are used for this purpose, one of which is shown in Fig. 48.

Either 9 or 18 gm of cream is weighed into the counterpoised test bottles. Fat is determined by the Babcock method as used for milk, described on page 208, with the exception of the method for reading the position of the upper end of the fat column. First determine the value of the upper meniscus (in per cent) between the extreme upper and lower points of the curve. Add one-third of this value to the reading of the lowest part of the curve and consider this the final reading.[1]

The fat column may be read more easily by adding a light mineral oil which has been colored red with a vegetable pigment. There are several such preparations on the market under trade names such as "glymol," "alboline" and "red top." The use of a colored mineral oil was suggested after extensive investigation of various compounds.[2] If such an oil has practically the same surface tension as that of melted butterfat the surface dividing the two liquids is approximately plane. While the use of such a device for making easier readings has become quite extensive there seems to be some evidence to the effect that lower readings are obtained in this way.

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[1] HUNZIKER, *Ind. Exp. Sta. Bull.*, **145** (1910).

[2] *Ibid.*

Total solids, ash, total nitrogen and lactose are determined as with whole milk. A somewhat smaller sample (2 to 3 gm) is used for the total solids determination. The gravimetric method for lactose is preferred.

ICE CREAM

Determination of Fat: *Röse-Gottlieb Method.*—Weigh 4 gm of ice cream (melted by exposure to air, then thoroughly mixed) in a 50-cc beaker, add 3 cc of water and mix with a glass rod. Pour the mixture into a Röhrig tube and wash the beaker and rod, using 3 cc of water and adding the washings to the tube. Add 2 cc of concentrated ammonium hydroxide and after mixing thoroughly, heat in a water bath at 60° for 10 minutes. Add 10 cc of 95-per cent alcohol and continue as directed on page 206 for the determination of fat in milk.

BUTTER AND SUBSTITUTES

Butter, according to the Federal standards, is the clean, non-rancid product made by gathering, in any manner, the fat of fresh or ripened milk or cream into a mass containing also a small portion of other milk constituents, with or without salt, and which contains not less than 82.5 per cent of milk fat.[1] By acts of Congress, approved Aug. 2, 1886 and May 9, 1902, butter also may contain added coloring matter.

Adulteration.—Analysis of butter is usually made (*a*) to determine whether or not some foreign oil or fat has been partly or wholly substituted for butter fat, (*b*) to determine whether an excessive amount of milk or water has been incorporated or (*c*) to identify "process" butter (butter which has been steamed to correct rancidity). Adulteration with another fat or steaming rancid butter changes the microscopical appearance of the mixture from non-crystalline (as butter fat naturally exists) to crystalline, the added fat usually having been previously melted.

Preparation of Sample.—Butter is not usually of a homogeneous composition but it can be made approximately so by melting and shaking during solidification. If large quantities of butter are to be sampled, use a butter sampler. Place 500 gm or more of the sample in a wide-mouth glass stoppered bottle and warm gently until the entire mass is melted. Stopper and shake vigorously and continue to shake during cooling, to prevent the separation of fat and water. Preserve in a cool place.

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[1] *U. S. Dept. Agr. Chem. Bull.*, **64**, 37 (1920).

Moisture.—This is a variable quantity in butter, the moisture content ranging from 5 to 25 per cent, but it averages about 16 per cent. Most of the states have laws regulating the maximum amount allowed. For the determination, dry sand or asbestos is used in the evaporating dish to increase the effective surface and thus increase the rate of the drying, unless the dried butter sample is to be used for the indirect determination of fat, in which case the sample is placed in the clean dish. There is danger of oxidizing the fat if it is subjected to prolonged heating.

Determination of Moisture.—Place about 2 gm of butter in a dish having a flat bottom and containing asbestos fiber or sand, the dish and contents having been dried at 100°, cooled and weighed. Weigh accurately, then dry the fat for one hour at 100°, cool in the desiccator and weigh. Repeat the drying, cooling and weighing hourly until the weight is constant to the third decimal. Calculate the per cent of water in the sample. Preserve the dried sample for the fat determination.

Fat.—The fat may be determined either directly, as in milk analysis, or indirectly by weighing the solids left after extracting the fat with ether or petroleum ether.

Determination of Fat: *Direct Method.*—The sample of water-free fat obtained in the moisture determination is used. Remove the asbestos fiber or sand containing the fat from the aluminium dish to an alundum cup or paper capsule for the extraction apparatus of Fig. 41, using anhydrous ether to rinse out the last traces of fat. Place in the extraction apparatus and thoroughly extract with ether, free from alcohol and water. (See page 147 for details of the use of the extractor.) Recover the ether by distilling it on a steam bath or electric hot plate, cooling the vapor by means of a glass condenser. Dry the flask and fat at 100°, cool, and weigh. Repeat the drying, weighing each hour until the weight is constant to the third decimal. Calculate the per cent of fat in the butter.

Indirect Method.—Prepare a Gooch filter, dry at 100° and weigh. Use the sample of butter which was dried without asbestos or sand in the moisture determination. Dissolve this in anhydrous, alcohol-free ether or anhydrous petroleum ether and pass through the filter. Wash with the solvent until free from fat. Dry at 100° and weigh. Calculate the fat by difference.

Casein or Curd.—The amount of curd in good butter varies from 0.4 to 0.9 per cent. Any considerable amount of buttermilk left in the butter causes a rapid development of protein decomposition products which impair the flavor of the butter. The casein may be determined by the Kjeldahl or Gunning method as in milk, using about 5 gm of sample, or by burning the casein from the residue from the indirect fat determination, calculating the loss in weight as casein. The temperature is kept just below redness (to avoid volatilizing salt) until the residue is white. A muffle furnace, heated to 600°, is suitable for this ignition.

Salt.—Salt is added to improve the keeping quality and the taste of butter. The amount added ranges from 2 to 6 per cent. Salt is determined by adding a standard solution of silver nitrate to the water extract of the butter, using potassium chromate as an indicator. (See page 52, Part I.)

Determination of Salt.—In a small counterpoised beaker place about 10 gm of butter, secured from various parts of the prepared sample, and weigh to the third decimal place. Add about 20 cc of boiling water and when the butter is melted transfer to a small separatory funnel of about 50-cc capacity. Shake the mixture thoroughly and allow the fat to come to the top of the water. Draw off the water layer into a 250-cc volumetric flask, guarding carefully against the passing of any fat globules. Repeat the extraction with hot water ten to fifteen times. Cool the washings to 20°, dilute to the mark on the flask, mix thoroughly and pipette 25 cc of the liquid into a casserole for the determination of salt. Titrate with twentieth-normal silver nitrate solution, using 1 cc of neutral 5-per cent potassium chromate solution as indicator.

Examination of Butter Fat.—The composition of butter fat is quite different from that of other fats in that it contains a larger proportion of the glycerides of fatty acids of low molecular weight, particularly of butyric acid.

In the following table by Brown,[1] the average composition of butter fat is expressed in terms of the various glycerides, also of free acids obtainable by hydrolysis.

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[1] *J. Am. Chem. Soc.*, **21**, 807 (1899).

TABLE XVIII.—AVERAGE COMPOSITION OF BUTTER FAT

Acid	Glyceride, per cent	Acid		
		Per cent	Soluble* in water	Volatile with steam
Butyric, C_3H_7COOH	6.23	5.45	+	+
Caproic, $C_5H_{11}COOH$	2.32	2.09	Partly	+
Caprylic, $C_7H_{15}COOH$	0.53	0.49	–	+
Capric, $C_9H_{19}COOH$	0.34	0.32	–	+
Lauric, $C_{11}H_{23}COOH$	2.73	2.57	–	Partly
Myristic, $C_{13}H_{27}COOH$	10.44	9.89	–	–
Palmitic, $C_{15}H_{31}COOH$	40.51	38.61	–	–
Stearic, $C_{17}H_{35}COOH$	1.91	1.83	–	–
Oleic, $C_{17}H_{33}COOH$	33.95	32.50	–	–
Dihydroxystearic, $C_{17}H_{33}(OH)_2COOH$	1.04	1.00	–	–
Total	100.00	94.75		

* Solubility is at 15°, given in approximate terms.

Butter Substitutes.—The oleomargarine of commerce is usually composed of refined olein of beef fat (called "oleo oil"), churned up with neutral lard, milk and some butter fat, the latter two imparting a butter flavor to the product. Other oils often used are cottonseed, peanut and cocoanut oils. Salt is added and sometimes the oleomargarine is colored to make it more nearly resemble natural butter. It is to be expected that the composition of the various oleomargarines would vary greatly because of the variety of oils and fats used in their manufacture. Even the fat from any one source may vary somewhat depending upon the conditions under which it was produced. The use of cocoanut oil in the manufacture of the "nut" margarines is very common practice because cocoanut "oil" is a high grade edible fat, resembling butter in several ways. The fact that some of its constants are near to those of butter makes its detection in butter substitutes more difficult.

Cocoanut fat, like that of butter, has a fairly high per cent of volatile fatty acids and it melts at nearly the same temperature.

It differs from butter fat mainly in the larger proportion of the volatile fatty acids that are insoluble in water. These differences are used in its detection, especially the volatile insoluble fatty acids as indicated by the Polenske value (page 186), which is the number of cubic centimeters of tenth-normal base required to neutralize the insoluble volatile fatty acids obtained from 5 gm of fat. The Polenske value for butter is 1.5 to 3.0, for oleo oil 0.5 and for cocoanut fat about 17. The average Reichert-Meissl number for cocoanut fat is about 7, while that of butter fat is about 28. This makes it possible to distinguish (*a*) between butter and butter substitutes and (*b*) between oleomargarine and "nut" butters. The fatty acids of the glycerides of cocoanut fat are as follows:[1]

Table XIX.—Fatty Acids of Glycerides of Cocoanut Fat

Acid	Per cent	
Caproic	2	Volatile but largely insoluble
Caprylic	9	
Capric	10	
Lauric	45	Non-volatile
Myristic	20	
Stearic	5	
Oleic	2	
Palmitic	7	

The particular "constants" that are mentioned in the following table will be found of greatest value in identifying butter and butter substitutes. Of these the Reichert-Meissl number, the Polenske value and the soluble acid number are perhaps of first importance. Microscopic examination will serve to distinguish process butter, by the fact that fat crystals are to be found only in butter that has been remelted.

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[1] Elsdon, *Analyst*, **38,** 8 (1913).

TABLE XX.—CONSTANTS OF BUTTER AND COMMON SUBSTITUTES

	Butter fat	Oleomargarine*	Nut butter†
Iodine absorption number	26–38	52–65	35.72
Soluble acids	4.5	0.7	
Insoluble acids	87.5	95.5	
Reichert-Meissl number	24–32	0.8–1.0	5–6.5
Polenske value	1.5–3.0	Less than 1	10–18
Saponification number	220–233	195	225–250
Index of refraction	1.454	1.458	
Butyro refractometer reading	42.0	48.0	43.5

* A representative oleomargarine made from oleo oil, lard and milk.

† Made from peanut and cocoanut oils.

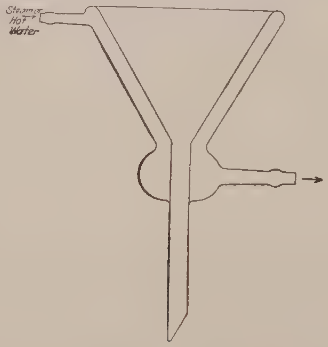

FIG. 50.—Steam or hot water funnel.

Preparation of Samples of Butter Fat.—Melt the butter at 60° and keep at this temperature for several hours, or until the curd and water have separated completely. Pour off the clear fat through a dry filter paper which is placed in a hot water or steam funnel (Fig. 50).

For the general methods to be used in the examination of butter fat refer to Chap. X, on fats, oils and waxes.

CHEESE

According to the Federal standard, cheese is the sound, solid and ripened product made from cream or milk by coagulating the casein with rennin or lactic acid, with or without the addition of ripening ferments. It contains, in the water free substance, not less than 50 per cent of fat.

Manufacture.—The action of the enzyme rennin is to change the casein, by hydrolysis, into albumoses and peptone and soluble paracasein. The calcium salts present in solution in the milk serum unite with the soluble paracasein to form an insoluble curd. The curd carries down not only the fat but also the greater part of the microorganisms contained in the milk. If these organisms are of a desirable kind they will produce a homogeneous curd and good cheese, otherwise a spongy curd and a cheese of poor consistency will be the result.

The table below gives the average composition of certain samples of four kinds of cheese.

TABLE XXI.—COMPOSITION OF CHEESE

Kind of cheese	Water	Fat	Ash	Proteins	Primary products of ripening	Secondary products of ripening
American "Cheddar"	34.2	33.7	3.8	25.2	...	...
Roquefort	31.2	33.2	6.0	27.6	...	...
Swiss	33.0	30.2	5.3	25.4	2.7	3.1
Gervais	44.8	36.7	2.9	12.7	1.9	0.8

Determination of Water.—Place about 15 gm of clean white sand in a flat aluminium dish, dry at 100° and weigh. Add about 3 gm of cheese and reweigh, quickly. More sand is needed if the cheese is very rich in fat. The mixture is then dried at 100° to constant weight and the per cent loss in weight calculated as moisture.

Determination of Ash and Salt.—Ignite, cool and weigh a covered porcelain crucible. Add about 15 gm of cheese, cover and reweigh. Uncover the crucible and drive off most of the moisture and volatile matter by carefully heating over a small flame. Place the uncovered crucible in a muffle furnace which is held at about 600° and through which air may circulate. Continue the heating until all carbon is burned. Cool in a desiccator and weigh. Calculate the per cent of ash.

After weighing the ash, add water, dilute to 250 cc in a volumetric flask and mix. Either 50 or 100 cc of this solution is taken for the chlorine determination as directed on page 223. Calculate as sodium chloride. The residue insoluble in water may be dissolved in a small amount of dilute hydrochloric acid, diluted to 250 cc and calcium and phosphorus determined in aliquot parts of this solution, if desired.

Determination of Fat.—Place a mixture of equal parts of anhydrous copper sulphate (dried at 225°) and pure dry sand in an alundum cup and fill to a depth of about 5 cm, packing loosely. Cover the upper surface of material with a layer of asbestos. Place on this 2 to 5 gm of sample and extract with anhydrous ether for 5 hours in a continuous extraction apparatus (Fig. 41, page 146). Remove the cheese to a mortar and grind it with the sand to a fine powder, return the mixed cheese and sand to the extraction tube, wash the mortar with ether, add the washings to the tube and continue the extraction for at least 10 hours.

Determination of Total Nitrogen.—Determine nitrogen as directed on page 151, using about 2 gm of cheese, and multiply the percent of nitrogen by 6.38 to obtain the per cent of protein.

Determination of Acidity.—To 10 gm of finely divided cheese add water at a temperature of 40° until the volume equals 105 cc. This allows 5 cc for the volume of the cheese. Shake vigorously and filter. Titrate 25-cc portions of the filtrate, representing 2.5 gm of sample, with tenth-normal sodium hydroxide using phenolphthalein as an indicator. Express the result in terms of lactic acid.

Detection of Coloring Matters in Butter or Cheese: (1) *Oil-soluble Dyes.*—Prepare an alcoholic solution of the oil-soluble dye by one of the following methods, which is to be applied to the oil or fat obtained by extraction with ether or gasoline.

(*a*) Shake the oil or melted fat with an equal volume of 90-per cent alcohol. The alcohol after separation will contain aniline yellow, "butter yellow," aminoazotoluene and auramine, or such of these as may be present.

(*b*) Dilute 20 to 200 gm (according to the intensity of color) of the oil or melted fat with two volumes of gasoline and shake out successively with 4-per cent potassium or sodium hydroxide solution, 15-per cent hydrochloric acid, and phosphoric-sulphuric acid mixture, prepared by mixing 85-per cent phosphoric acid with about 20 per cent by volume of concentrated sulphuric acid.

The dilute base extracts Sudan G and annatto. The dilute hydrochloric acid extracts aniline yellow, aminoazotoluene and "butter yellow," the first two forming orange-red, the latter cherry-red solutions in this solvent. The phosphoric acid mixture is necessary for the extraction of Sudan I, Sudan II, Sudan III and Sudan IV. Benzeneazo-β-naphthylamin and homologues also come in this group, though they readily undergo chemical changes in the strongly acid mixtures. The procedure is not very suitable in the presence of auramine but this dye is seldom found in oils.

Neutralize the alkaline and dilute hydrochloric acid solutions. Dilute the phosphoric acid mixture and partially neutralize, cooling the liquid during the operation. Extract the dyes from the neutral solutions by shaking with ether or gasoline.

For the direct dyeing test use the alcoholic solution obtained in (*a*). Evaporate to dryness the ether or gasoline solutions, obtained as directed in (*b*), and dissolve the residue in 10 to 20 cc of 95-per cent alcohol. To the alcoholic solution add some strands of white silk and a little water and evaporate on the steam bath until the alcohol has been removed or until the dye is taken up by the silk. The dyeing test is sometimes unsatisfactory, and in all cases a small portion of the alcoholic solution should be tested by treating with an equal volume of hydrochloric acid or stannous chloride solution. The common oil-soluble coal-tar dyes are rendered more red or blue by the acid and are decolorized by the reducing agent. Most of the natural coloring matters become slightly paler with the acid and are little changed by the stannous chloride solution.

(2) *Annatto Coloring Matter.*—Pour on a moistened filter a basic solution of the color obtained by shaking out the oil or melted and filtered fat with warm, dilute sodium hydroxide solution. If annatto is present the filter paper will absorb the color so that when washed with a gentle stream of water it will remain dyed a straw color. Dry the filter and add a drop of stannous chloride solution. If the color changes to pink the presence of annatto is confirmed.

CHAPTER XII

SOILS

A soil analysis is made to find out the extent and distribution of plant food elements and thus to determine which elements are the limiting factors in crop production. The term "soil fertility" is often used to express this relation and this is understood to mean the crop producing power of any soil under specified climatic conditions. "Fertility" is really an indefinite term as the property indicated is the resultant of many forces which are frequently opposed to each other in their action.

Total and Acid-soluble Material.—The analysis of soils with a view to measuring their fertility and studying their geological origin has received much attention in recent years. It was formerly thought, and it is still held to some extent, that the fertility can best be measured by extracting the soils with strong acids, thus obtaining an invoice of the total plant food contained. But by this method there is left some available potassium and a mass of substances of unknown composition, which yet need to be determined in order to have complete information concerning the geological origin of the soil. On this account the value of many early analyses is being called into question and at present it is regarded as desirable that the total constituents of the soil should be determined. The complete examination of a soil involves its study from chemical, physical and biological points of view. The chemical phases of this subject will be given most attention here.

Soil Constituents.—Soil has been defined as that portion of the earth's surface, climatic conditions being favorable, which makes possible complete growth and development of plants. Ordinarily soils are made up of mixtures of organic matter, rock at various stages of disintegration, water, gases and bacteria. The great mass of this material is not directly essential to the growth of plants but aids in holding moisture and making a medium in which the roots may anchor themselves.

Classification of Plant Food Elements in the Soil.—The plant food elements of the soil occur principally as follows:

1. Nitrogen is found in soils as a constituent of organic matter, nitrates, ammonium salts and amino acids.
2. Phosphorus is present in organic forms or combined with calcium, iron or aluminium as phosphate.
3. Potassium is widely distributed in all soils of granitic origin. It is combined with silica as silicates in granite, orthoclase and mica.
4. Calcium is found as carbonate and silicate, as sulphate in gypsum, as a constituent of rock phosphate and in organic forms.
5. Sulphur occurs combined with calcium as gypsum. It is quite deficient in some soils.
6. Iron and manganese are found as oxides and silicates.
7. Magnesium is associated with calcium as dolomitic limestone, also as silicates.

Aluminium, sodium and silicon are probably non-essential to plant growth.

Value of Soil Analysis.—There has been much discussion concerning the adequacy of soil analysis as a means of measuring soil fertility. Many writers confuse the narrow purpose of simply determining the plant food immediately available with the broader one of obtaining an extensive knowledge of the total plant food supply and a determination of the possible geological origin of the soil in order to plan better for permanent improvement. The value of the analysis is expressed by Hopkins[1] as follows:

> "The chief value of a chemical analysis is to serve as an absolute foundation upon which methods of soil treatment can safely be based for the adoption of a system of permanent soil enrichment, not for one crop or for one year only but for progressive improvement."

The Ohio Station has shown[2] how accurately the excesses and deficiencies may be measured by analysis when good and poor methods of agriculture have been practiced for a period of fifteen years. There is no other tool which compares with it for this purpose and as an aid in unlocking soil secrets. The value of soil analysis in the determination of geological origin has been considerably underestimated, although few data are at hand to aid in such an interpretation.

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[1] "Soil Fertility and Permanent Agriculture," p. 568.

[2] *Ohio Exp. Sta. Bull.*, **261** (1913).

Soil Classification Based upon Mode of Formation.—For convenience in the study of soils they may be divided according to manner of formation into eight groups[1] as follows: *Cumulose* soils are chiefly deposits of vegetation in various stages of decay. *Residual* soils—unmoved from the rocks from which they were formed. *Loess*—residue deposited as dust carried by wind. *Glacial* soils are deposits which have developed from glacial action. *Colluvial* soils are deposits which have been moved down hill by gravity. *Alluvial* soils consist of residues deposited from flowing water. *Marine* soils are formed by deposits carried into seas. *Lacustrine* soils are formed by deposits carried into lakes.

Classification Based upon Composition.—It is to be expected that soils whose origin is so different as is noted above would vary greatly in chemical analysis. Ames has made a study (unpublished work) of the relation between soil type and composition, considering at the same time the geological formation. The chief differences observed were with respect to the calcium carbonate, total organic matter and nitrogen content. For example the soils of limestone origin, as compared with those from sandstone and shales, contained larger amounts of calcium and magnesium. In many cases these larger amounts of calcium and magnesium are accompanied by larger amounts of phosphorus, organic matter and nitrogen. It is evident that if only disintegrating forces had been active the soil particles would be of the same general composition as the original rock. However, many agencies tend to change this original material until only the most resistant minerals remain.[2]

The Report.—Determinations of the inorganic constituents of soil are usually reported in terms of their most stable compounds or of their oxides although nitrogen is reported as the element. The determination is usually made on an air-dried sample. Besides reporting results as per cents on this basis it is sometimes desirable to report the amount per acre (2,000,000 lb. is considered as the approximate weight of a loam soil, 6⅔ in. deep over an acre of land, while 1,000,000 lb. is taken as the average weight of a muck soil over the same area).

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[1] TROWBRIDGE, *J. Geol.*, **22**, 420 (1911).

[2] See also *N. C. Exp. Sta. Tech. Bull.*, **9** (1914).

Analytical Methods.—The chemical methods for studying the soil may be considered under the following heads:

(*a*) Complete analysis.
(*b*) Potential plant food.
(*c*) Available plant food.

Complete Analysis.—The inorganic analysis is usually made by fusing the soil with alkali carbonates, the insoluble silicates forming alkali silicates which can be dissolved in hydrochloric acid. From this solution the total inorganic constituents, with the exception of carbon dioxide, sulphur, sodium and potassium, may be determined. The latter group, as well as moisture, nitrogen, phosphorus, and organic matter, are determined in separate samples.

Potential Plant Food.—This is separated by digesting the soil in hydrochloric acid of constant boiling point (specific gravity 1.115, containing about 23 per cent of HCl), using the ratio of 1 part of soil to 10 of acid, thus effecting the solution or partial decomposition of soil minerals. This was formerly the official method.

Available Plant Food.—This is the part of the total supply which is immediately available to plants. There are many natural agencies tending to make plant food available, such as bacterial action, plant decay and root acidity, and it is difficult to determine the part played by any one, especially by dilute acids. After making an extensive study of the acidity of various plant roots it was suggested by Dyer in 1894 that solubility in a 1-per cent solution of citric acid most nearly measured the true availability, as indicated by the ability of growing plants to absorb material from the soil. Various other solvents have been tried, such as distilled water, carbonated distilled water, acetic acid, aspartic acid and fifth-normal hydrochloric and nitric acids. The latter seems to give more consistent results on many soils than does citric acid. However, all laboratory results obtained by the use of weak solvents are only approximations to the action of natural solvents in the soil.

Choosing Samples.—In choosing soil samples it is very important to secure representative ones. The sampling should be done when the ground is dry enough to plow. An area should be selected such that the soil is typical with respect to texture and color. Note should be made of any available information concerning the geology of the area, original timber of the land, the present productivity, or any peculiarities in location which may aid in interpreting its analysis. The surface accumulations of such materials as decaying grass should be removed and the borings for samples then made with a soil auger or other soil tube. Composite samples are taken from different depths as follows: (*a*) surface to 6 in., (*b*) 6 to 20 in., (*c*) 20 to 40 in. For each depth ten to fifteen borings are taken and well mixed. About a pint of soil from each depth should finally be preserved. Sample (*c*) need not be mixed with as great care as are samples (*a*) and (*b*) since it is not usually taken for analytical purposes but for obtaining some insight into the physical nature of the subsoil, drought resistance and drainage depending to some extent upon the nature of substrata. The borings are placed in clean cloth sacks in the field and immediately sent to the laboratory. Here they are dried and later ground for analysis.

Preparation of Samples.—Spread the samples on paper or in shallow pans in a dry place, in clean air, and allow to remain until apparently dry. Pulverize lumps and divide each sample into two fractions by use of a 4-mesh sieve. The stone remaining in the sieve is weighed and its per cent of the total is calculated. Grind the finer soil portion in a porcelain pebble mill or other pulverizer until it will pass a 40-mesh sieve. Mix thoroughly and then grind about 100 gm of this sample until it will pass a 100-mesh sieve. Riffles of different sizes may be used for sampling, or rolling on paper or oilcloth may be employed. (See the discussion of sampling, Part I, pages 17 to 22.) The samples should be placed in stoppered bottles and carefully labeled.

Moisture.—The proportion of moisture in air-dried soil depends largely upon the proportion of organic matter. The water-holding power of soil is of great significance from a practical standpoint.

Determination of Moisture.—Weigh accurately 5 gm of finely pulverized 100-mesh soil into a flat porcelain crucible about 4 cm in diameter and provided with a glass cover. Remove the cover and dry the sample at 110° for five hours. Cover and cool the crucible in a desiccator and then weigh. Preserve the dried sample for the determination of volatile matter. Calculate the per cent of moisture in the prepared soil.

Optimum Moisture of Soils.—The water-holding capacity of a soil depends upon its content of organic matter and its structure. The amount of water which just permits a soil to crumble is considered the optimum. This is about one-third of its total water-holding capacity.

Approximate Determination of Optimum Moisture Content of Soils.—Weigh three 25-gm portions of the 40-mesh air-dried soil and place them in 200-cc beakers or wide-mouth bottles. Add to the three portions, 5, 6 and 7 cc of water, respectively. Cover the bottles with watch glasses and allow to stand for two days. Remove the soil and see if any sample is wet enough to form balls. If not, repeat the experiment with modified quantities of water. The optimum moisture should be just a little less than this amount.

Total Nitrogen.—The relative amount of nitrogen in soils varies greatly, although it is usually approximately in proportion to the organic matter. A soil in Manitoba is reported to contain as high as 20,100 lb. per acre (1.005 per cent) while a sample from the "Jack Pine" plains of Michigan is said to contain only 740 lb. per acre of 2,000,000 lb. (0.0037 per cent) of soil.

Nitrogen is one of the most important of all elements in the soil. It is absolutely essential to plant existence and it cannot be taken from the abundant supply of the air by the plant itself. Certain forms of soil bacteria cause the fixation of this elementary nitrogen in the form of nitrates, which can then be utilized by the plant. The chief purpose of nitrogen in plant economy is to provide for the construction of protein by the plant. The deep green color of plant leaves is often an indication of an abundance of available nitrogen.

Determination of Total Nitrogen.—Place 10 gm (5 gm if a muck soil) of 40-mesh soil and 30 cc of concentrated sulphuric acid in a 500-cc Kjeldahl flask. Proceed as described on page 151, and following. Calculate the per cent of nitrogen.

Nitrate Nitrogen.—The amount of nitrate nitrogen present in a soil depends mainly upon the amount and kind of vegetation, and upon the degree of compactness, the temperature and the water content of the soil. The most favorable temperature seems to be about 35° and the most favorable water content is one-third of its saturation. These factors largely determine whether or not a soil is suitable for bacterial development.

The amount of nitrate present in a soil at any one time is seldom very large, ranging from zero to 1000 lb. per acre (0.05 per cent). It is difficult to find more than a trace of nitrate nitrogen in soil just under an old sod whereas in some western soils nitrates have accumulated in such amounts as to interfere with plant growth.[1]

The phenoldisulphonic acid method is used for the determination of nitrates, the following equations representing the reactions:

$$H_2SO_4 + 2KNO_3 \rightarrow 2HNO_3 + K_2SO_4; \qquad (1)$$

$$\underset{\text{Phenoldisulphonic acid}}{C_6H_3OH(SO_3H)_2} + HNO_3 \rightarrow \underset{\text{Nitrophenoldisulphonic acid}}{{}_6CH_2OH \cdot NO_2(SO_3H)_2} + H_2O; \qquad (2)$$

$$C_6H_2OH \cdot NO_2(SO_3H)_2 + 3NH_4OH \rightarrow C_6H_2ONH_4(SO_3NH_4)_2NO_2 + 3H_2O. \qquad (3)$$

The ammonium salt of nitrophenoldisulphonic acid, thus formed, is intensely yellow and the color so produced is compared with that formed from a standard nitrate solution.

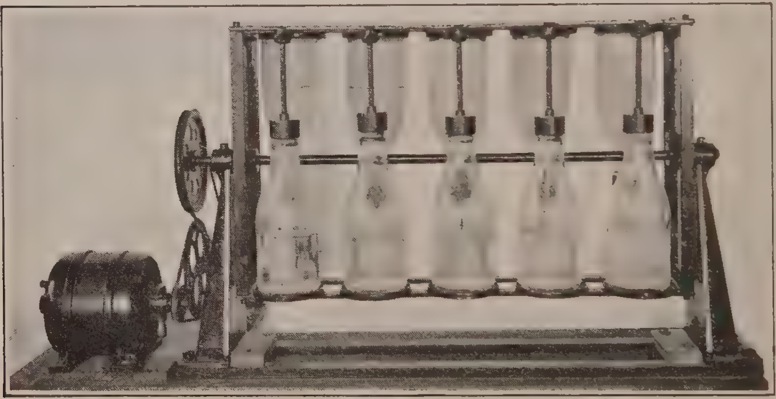

FIG. 51.—Mixing machine.

Determination of Nitrate Nitrogen.—Prepare the following reagents:

(*a*) *Phenoldisulphonic Acid.*—Mix 30 gm of pure crystallized phenol with 370 gm of concentrated sulphuric acid. Immerse the flask in boiling water for six hours. When cool store in an amber colored bottle. A smaller quantity of the solution may be made, if desired.

(*b*) *Standard Color Solution.*—Prepare a standard solution of potassium nitrate by dissolving 0.7215 gm of dried pure potassium nitrate in distilled water and diluting to 1 liter. Each cubic centimeter of this solution will contain 0.1 mg of nitrogen. Pipette 10 cc of this solution into a dish and evaporate to dryness over a steam bath. Cool and moisten the dry nitrate with 2 cc of phenoldisulphonic acid, rubbing together with a glass rod.

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[1] *Colo. Exp. Sta. Bull.*, **178** (1911).

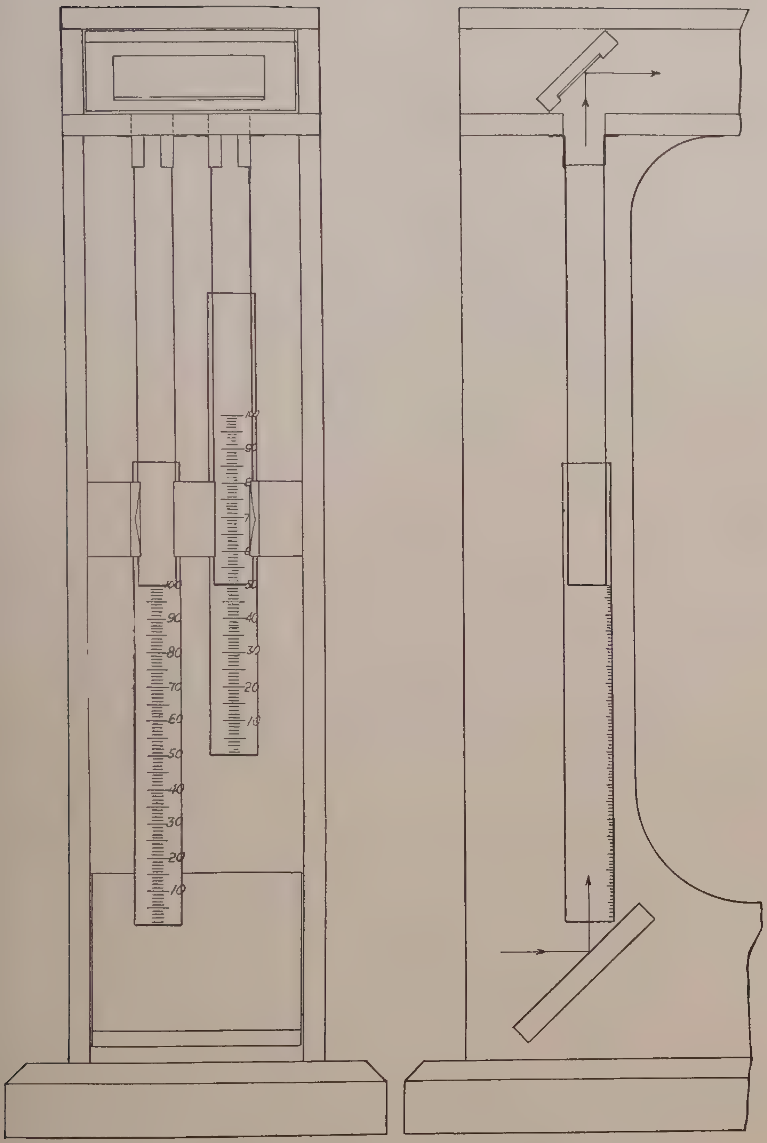
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FIG. 52.—Schreiner color comparator.

After 5 minutes dissolve and dilute to 1000 cc in a volumetric flask. This makes a permanent color standard, 1 cc of which will contain 0.001 mg of nitrate nitrogen.

Place two 100-gm samples of 40-mesh soil and 5 gm of calcium hydroxide (to aid in securing a clear solution) in salt mouth or shaker bottles and add 400 cc of nitrate-free distilled water (tested as below) to each bottle. Mix in a machine for 30 minutes and then remove the bottles and let stand over night. Pipette 10 cc or more of the clear, supernatant solution into a 8-cm porcelain evaporating dish and evaporate to dryness on a steam bath. Remove from the steam bath as soon as dry, cool, add 2 cc of phenoldisulphonic acid and mix well with the aid of a glass rod. After the acid has stood in contact with the residue for 15 minutes add 5 cc of cold distilled water, stir and add enough ammonium hydroxide (1 to 1) to produce a permanent yellow color. The standard (a suitable measured quantity of which has been made basic with ammonium hydroxide in the same manner as the unknown) is rinsed into a cylinder for a colorimeter, such as that illustrated in Fig. 52, and diluted to the 100-mm mark. Rinse the unknown into another tube and dilute to the 100-mm line, provided that the color of this solution is not over two-thirds as intense as that of the standard. Place both tubes in the colorimeter and move the tube containing the more intense color up or down until the intensities of color in the two are equal. The nitrogen concentrations are inversely as the lengths of column equivalent in intensity of color. Take three readings on each sample and from these calculate the per cent of nitrate nitrogen in the sample.

Ammonia.—The amount of ammonia nitrogen in soils is usually very small, although in certain swamps it is present in considerable quantities as ammonium salts. Such plants as rice, which grow in water, secure considerable nitrogen in the form of ammonia or of nitrogenous organic decomposition products. Among these compounds are amino acids, *e.g.*, arginine and glycocoll.

The chemistry of a possible mode of ammonia production from amino acids may be represented by the following equations:

$$\underset{\text{Amino acid}}{RCHNH_2COOH} + O_2 \rightarrow \underset{\text{Fatty acid}}{RCOOH} + CO_2 + NH_3; \quad (1)$$

$$RCHNH_2COOH + H_2O \rightarrow RCHOHCOOH + NH_3. \quad (2)$$

Thus an amino acid when oxidized or hydrolized produces ammonia as an end product.

Determination of Ammonia.—Place 25-gm samples of soil, together with 5 gm of sodium carbonate, in aeration flasks (Fig. 53), add three drops of light hydrocarbon oil (to prevent frothing) and 100 cc of boiled distilled water to each flask. Connect the flask with a wash bottle containing 25 cc of tenth-normal sulphuric acid and also to another wash bottle containing 10-per cent sulphuric acid to free the incoming air from all traces of ammonia gas, as shown. Aerate by suction for two hours, the air and gases passing from the aeration flask to the bottle containing tenth-normal acid. At the end of this period, titrate the standard acid with tenth-normal base, using methyl red as indicator. From the volume of standard acid neutralized by the ammonia obtained from the soil calculate the per cent of ammonia in the soil sample.

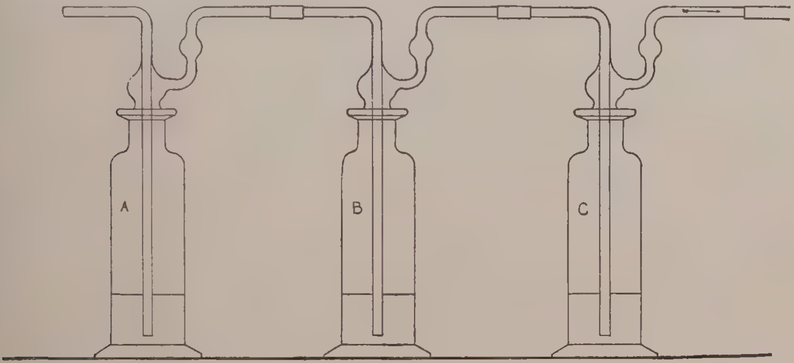

FIG. 53.—Aeration apparatus.

Nitrification.—A productive soil is not simply a dead medium in which the plant can fix itself and from which it can get food by processes of solution and diffusion; on the contrary it is teeming with living organisms which bring about changes difficult to duplicate in the laboratory. By their activity much food which otherwise might not be used is made available to the plant. Two important organisms have been isolated. One, called "nitrosococcus," causes oxidation of ammonia to nitrites and the other, "nitrobacter," causes oxidation of nitrites to nitrates, according to the following reactions:

$$2NH_3 + 3O_2 \rightarrow 2HNO_2 + 2H_2O, \qquad (1)$$

$$2HNO_2 + O_2 \rightarrow 2HNO_3. \qquad (2)$$

Measurement of Nitrification.—Prepare 450 cc of an approximately fifth-normal solution of ammonium sulphate in a 500-cc volumetric flask and add 0.3 gm of dipotassium phosphate, 0.5 gm of calcium carbonate, and 2.0 gm of a fresh sample of a fertile soil. The purpose of dipotassium phosphate is to furnish food for the growth of bacteria. The nitrifying bacteria in the soil will convert the ammonia nitrogen to nitrate nitrogen and the calcium carbonate serves to neutralize the nitric acid formed. Mix thoroughly. Dilute to the mark and determine the per cent of ammonia nitrogen in 100 cc by placing the solution in a Kjeldahl flask, adding 15 cc of 10-per cent potassium hydroxide and distilling the ammonia into 25 cc of fifth-normal hydrochloric acid, using a tin condenser.

Place 200 cc (or enough to cover half the sand) of the ammonium sulphate culture solution, prepared as above, on clean white sand in a percolator (Fig. 54). Cover the percolator with a watch glass. Keep in a dark place for three or four weeks, then drain out all liquid and rinse the sand with about 200 cc of distilled water (ammonia-free), using about 50 cc portions at a time. Make the solution up to 500 cc in a volumetric flask, mix well and determine the amount of ammonia nitrogen in an aliquot part by the distillation method, as above described. The difference between the amount present at the beginning and at the end will represent the amount converted to nitrate. Calculate the per cent of nitrogen which has been changed from ammonium sulphate to a nitrate. This gives an estimate of the activity of nitrifying bacteria in the soil.

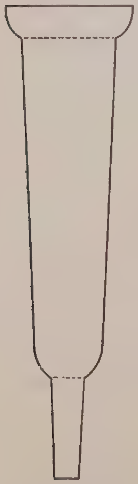

Fig. 54. Percolator.

Denitrification.—Certain bacteria (*B. denitrificans alpha*, also *beta*) have the power, under appropriate conditions, to reduce ammonia, nitrites and nitrates to the form of elementary nitrogen. This is usually brought about in a water-logged soil or in the presence of an excess of nitrogenous organic matter. The amount of released elementary nitrogen may be measured and the bacterial activity noted.

Demonstration of Denitrification.—In a 250-cc wide mouth flask or bottle place 20 gm of horse manure and add 100 cc of water containing 1 gm of potassium nitrate. Fill the bottle with water and close the mouth with a rubber stopper connected with a delivery tube. The tube is inserted into a 500-cc graduated cylinder which has been previously filled with a 5-per cent sodium hydroxide solution and inverted into a 1000-cc beaker containing about 300 cc of water. The method of assembling the apparatus is shown in Fig. 55. After standing 24 hours at about 35° a mixture of carbon dioxide and nitrogen will begin to be produced. The former will be absorbed by the sodium hydroxide while the latter will be caught in the cylinder and can be measured. Calculate the per cent of denitrification of the nitrate added.

Phosphorus.—Phosphorus is present in all soils, usually in small amounts, varying from 300 to 5000 lb. per acre of 2,000,000 lb. of soil (0.015 to 0.25 per cent). The plant demand for phosphorus is large, as crops remove from 5 to 30 lb. per acre annually. It occurs in the soil chiefly as apatite (calcium fluorophosphate) and, to some extent, in organic forms.

The most pronounced effect of phosphorus upon the plant is noted in the greatly increased development of lateral and fibrous roots. This feature is of much importance in clay soils, especially as it induces the formation of an extensive system of roots, thus enabling the plant more successfully to withstand drouth. A deficiency of phosphorus is often shown by late maturity of crops and, in the case of cereals, in the lack of good grain development.

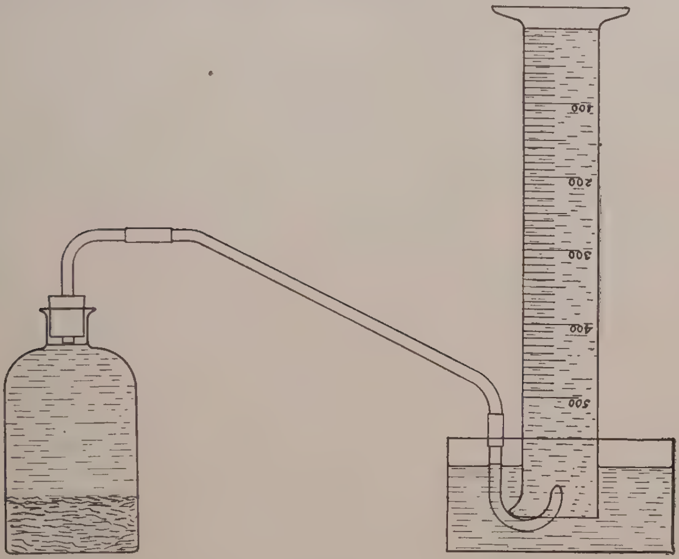

Fig. 55.—Denitrification apparatus.

Before phosphorus in soil can be determined it is necessary to remove organic matter, oxidizing phosphorus so held to phosphoric acid, and to bring the phosphorus of both organic and inorganic matter into solution. The methods now in use for this purpose are (*a*) oxidation of organic matter by heating with sodium peroxide, with subsequent solution of phosphates by hydrochloric acid, (*b*) a procedure similar to (*a*) but substituting magnesium nitrate for sodium peroxide, and (*c*) oxidation of organic matter and solution of phosphates by heating with concentrated sulphuric acid, with or without the addition of a catalyzer. From the solution produced by any of these methods phosphorus is precipitated with a molybdate solution as ammonium phosphomolybdate. The precipitate is either dissolved in standard base and the excess of the latter titrated, or it is dissolved in ammonium hydroxide and the phosphorus precipitated as magnesium ammonium phosphate, ignited to magnesium pyrophosphate and this weighed. The principles of these methods are discussed in Part I, pages 88 and 91.

Determination of Phosphorus.—Use one of the following methods for obtaining the phosphate solution:

(*a*) Place 10 gm of sodium peroxide in an iron crucible, add 5 gm of the soil and mix thoroughly by means of a glass rod. If the soil contains only a small proportion of organic matter add 0.5 gm of starch and mix as before. The starch will hasten the action. Heat the mixture by applying the flame of a burner directly upon the surface of the charge and the sides of the crucible until the action starts. Cover the crucible until the action is over and continue heating at a temperature of dull redness for 15 minutes. The residue in the crucible should not be fused. Transfer the charge to a 250-cc beaker with about 150 cc of water; add hydrochloric acid until acid to methyl red and boil. Cool, rinse into a 250-cc volumetric flask, dilute to the mark and mix. If the action has taken place properly there should be no particles of undecomposed soil in the bottom of the flask, although the solution will usually be turbid from silicic acid.

(*b*) Place 5 gm of soil in a 50-cc porcelain crucible and moisten with 5 cc of 50-per cent magnesium nitrate solution. Evaporate to dryness on the steam bath and ignite at dull redness. Let the crucible cool and add 5 cc of water and 10 cc of concentrated hydrochloric acid, then cover and heat on the steam bath for two hours. Stir several times while digesting. Transfer the contents of the crucible to a 250-cc volumetric flask, cool to room temperature, dilute to the mark and mix well.

(*c*) Place 5 gm of soil in a 500-cc Kjeldahl flask and digest with 30 cc of concentrated sulphuric acid and 0.5 gm of mercuric oxide until the carbonaceous matter has been oxidized. Cool to room temperature (do not place the flask in cold water until it has cooled somewhat in air), then add 100 cc of water, 5 cc of concentrated hydrochloric acid and 2 cc of concentrated nitric acid. Boil for 5 minutes, cool, dilute to 250 cc in a volumetric flask and mix well.

Filter the phosphate solution through a dry folded filter until the filtrate is no longer turbid. By means of a pipette or volumetric flask measure 100 cc of the clear solution and deliver into a 10-cm porcelain dish. Evaporate on the steam bath to dryness, take up with 5 cc of hydrochloric acid and an equal amount of water, filter to remove silica and wash. From this point proceed as directed in Part I, page 90, beginning with "Add ammonium hydroxide until a slight precipitate of hydroxides. . . . .," or as on page 92, beginning with "Add ammonium hydroxide until a slight precipitate persists. . . . ."

Potassium and Sodium.—Potassium is essential to plant growth and it is present in most soils in sufficient amounts to meet the plant needs, but only partly in an available form, it being very gradually changed to soluble potassium carbonate by action of carbonic acid upon orthoclase, which is nearly insoluble and thus not readily available to plants. Sandy soil often contains less than 0.1 per cent of acid-soluble potassium, sandy loams from 0.1 to 0.3 per cent, loams from 0.3 to 0.45 per cent and heavy clays 0.45 to 0.8 per cent.

Potassium functions notably in the photosynthesis and movement of starch within the plant. Lack of starch formation and movement is one cause of shriveled and sterile grain. Another effect of a lack of potassium is to make the plant less resistant to disease. This may be said of a plant suffering from any plant food deficiency but it seems to be especially true in the case of potassium.

Sodium is not of great importance in plant nutrition. It is credited with delaying potassium starvation but it will not entirely prevent this condition.

The method generally used for decomposition of insoluble minerals, preliminary to the determination of potassium and sodium, is the J. Lawrence Smith method. It is based upon the action of calcium chloride (formed from calcium carbonate and ammonium chloride) upon complex silicates at temperatures between 800° and 900°. Sodium and potassium chlorides, as well as silicate of calcium, are formed. The reaction taking place between orthoclase, calcium carbonate and ammonium chloride may be represented as follows:

$$2KAlSi_3O_8 + 6CaCO_3 + 2NH_4Cl \rightarrow 2KCl + Al_2O_3 + 6CaSiO_3 + H_2O + 2NH_3 + 6CO_2.$$

A long platinum crucible (Fig. 56) is preferable for the decomposition but an iron or nickel[1] crucible of 50-cc capacity may be used. Such base metal crucibles deteriorate rapidly when used in this way.

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[1] Walker, *J. Ind. Eng. Chem.*, **11**, 1139 (1919).

In the solution of salts finally obtained potassium may be separated from sodium by the chlorplatinate or the perchlorate method or it may be precipitated as potassium sodium cobaltinitrite and a volumetric method used for its determination. The accuracy of the various methods is in the order named, although the high cost of platinum is a great obstacle to the continued use of the chlorplatinate method and its recovery from residues involves considerable expense and loss of metal in each operation.

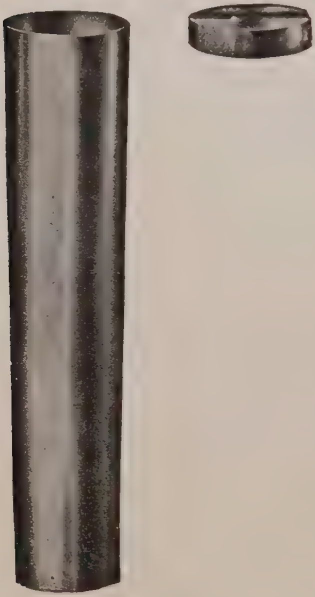

Fig. 56.—J. L. Smith crucible.

Chlorplatinate Method.—This consists in the precipitation of potassium chlorplatinate from an alcoholic solution by chlorplatinic acid:

$$2\mathrm{KCl} + \mathrm{H_2PtCl_6} \rightarrow \mathrm{K_2PtCl_6} + 2\mathrm{HCl}.$$

Sodium chlorplatinate is soluble in alcohol and this fact is used in its separation from potassium. Ammonium chlorplatinate, also, is only slightly soluble in alcohol. It is therefore necessary that ammonium salts be expelled by heating, before the reagent is added, and that the work be done in a room free from ammonia.

Decomposition of Soil Sample.—Grind in an agate mortar 0.5 gm of soil, accurately weighed, with 0.5 gm of ammonium chloride. When thoroughly mixed, add 4 gm of precipitated calcium carbonate and mix well by grinding. Place about 2 gm of calcium carbonate in the bottom of the crucible then add the ground mixture from the mortar and rinse the latter with about 0.5 gm of calcium carbonate. Brush the mortar well and add any traces of material to the charge in the crucible. Settle the mixture well by tapping gently, place the crucible in a hole in an asbestos board and heat in such a way that only the lower portion is reddened. After ammonia ceases to escape, turn on the full heat of the burner to all but the upper portion of the crucible and continue the heating for 45 minutes. The crucible should be red hot. The entire heating may be done more conveniently by placing the asbestos carrying the crucible over the top of a small electric furnace of crucible type, the lower portion of the crucible being in the furnace. The temperature of the latter is gradually raised to 800–900°, as shown by a pyrometer.

After the crucible has been cooled transfer the contents to a 300-cc porcelain dish, add sufficient hot water to cover the semi-fused mass, heat to boiling and let stand until the whole mass is completely slaked. Some samples are difficult to slake, due usually to heating to too high a temperature or to the presence of too little calcium carbonate in the mixture. These require digesting for some time on a steam bath; or the solution and residue may be placed in a porcelain dish and ground gently with an agate pestle. Filter the solution containing the disintegrated mass, collecting the filtrate in a 400-cc Pyrex beaker. Macerate the residue in a mortar, rinse several times with boiling water and finally filter and wash with boiling water until about 350 cc of filtrate has been collected.

To the filtrate add enough ammonium hydroxide to make basic, then ammonium carbonate to precipitate calcium. Heat to boiling then filter into a platinum dish, evaporate to dryness on the steam bath and heat to dull redness to expel most of the ammonium salts. Dissolve the residue in 5 cc of hot water. If any insoluble residue remains, repeat the addition of ammonium hydroxide and carbonate, filter through a small paper, wash the paper with hot water, add 1 cc of dilute hydrochloric acid to the filtrate and evaporate filtrate and washings in a platinum dish. Heat to dull redness for a short time to expel ammonium salts. This residue of potassium and sodium chlorides is ready for the determination of potassium.

Determination of Potassium: *Chlorplatinate Method.*—(To be performed in an atmosphere which is free from ammonia.) Dissolve the residue of potassium and sodium chlorides, obtained as above directed, in 50 cc of hot water and then add chlorplatinic acid (containing 10 per cent of platinum or 26.5 per cent of chlorplatinic acid crystals), using about 1 cc more than the theoretical amount, calculated upon the assumption that the chloride residue was all potassium chloride. Evaporate on the steam bath to a thick paste but not to dryness, cool and add 50 cc of 80-per cent alcohol, stir up the solid matter and allow to stand, covered, for 30 minutes.

If the liquid is not visibly colored too little reagent has been used. In this case new samples should be taken and the quantity of chlorplatinic acid increased. Filter and wash the precipitate thoroughly with 80-per cent alcohol, washing several times after the washings pass through colorless. The wash bottle should be provided with ground-glass joints so that no rubber will come into contact with the alcohol. Remove the filtrate and washings, pouring these into the bottle provided for platinum waste residues, and wash the precipitate again, thoroughly, with 80-per cent alcohol, using particular care in washing the upper part of the paper. Wash until only a faint turbidity is produced by the addition of a drop of silver nitrate solution to the last washings.

Drain most of the alcohol from the paper (or see next paragraph), slip the latter out of the funnel and dry in the oven at 100°. Place a weighed porcelain crucible upon a piece of glazed paper, remove most of the precipitate to the crucible, brushing up any particles that may have fallen upon the glazed paper, and then replace the paper in the funnel. Place the crucible under the funnel and dissolve the remainder of the precipitate in the smallest amount of nearly boiling water, allowing the solution to run into the crucible. Evaporate to dryness on the steam bath, carefully wipe the outside of the crucible with a clean towel and dry for 30 minutes at 105°. Weigh and calculate the per cent of potassium in the soil.

Use of a Gooch or Alundum Crucible.—Proceed as above until ready to filter out the potassium chlorplatinate. Prepare two Gooch filters as directed on page 50, paying attention to the precautions suggested, and using strong suction in forming the asbestos felt; or wash alundum crucibles with hot water, using suction. Finally rinse the crucibles with alcohol, remove, wipe the outside and dry at 100° to 105° for 30 minutes or until the weight is constant. Weigh and replace in the holder. If Gooch crucibles were used, moisten the asbestos with one or two drops of alcohol before the suction pump is again turned on. Start the pump, then filter and wash the precipitate exactly as above directed. Remove the crucible, dry in the oven and weigh. Calculate potassium as before.

Recovery of Platinum from Waste and Preparation of Chlorplatinic Acid.[1]—Place the waste solutions in an evaporating dish having a capacity of 2 liters for each 100 gm of platinum and evaporate until most of the water has been expelled. Make basic with sodium hydroxide and add, stirring, sodium formate, either solid or in concentrated solution. A quantity of sodium formate equal to about half the weight of platinum to be reduced will be required. If foaming occurs, add more sodium hydroxide. Heat on the steam bath for one hour, stirring occasionally, then acidify with hydrochloric acid (25-per cent solution) stirring during the addition of acid.

Filter off the precipitated platinum on a soft paper, using suction. Wash twice with hot 2-per cent hydrochloric acid, then with hot water until free from acid. Separate the platinum from the paper, dry, ignite and weigh. Pour over the platinum in a porcelain dish five times its weight of 25-per cent hydrochloric acid, heat on the steam bath and add slowly 50-per cent nitric acid until no more gas is evolved. About 1 cc of nitric acid will be required for each gram of platinum.

After the platinum is in solution, add 10 cc of 25-per cent hydrochloric acid, evaporate to small volume and repeat this process twice. This reduces and eliminates nitric acid. Dilute with water and evaporate, two or three times, to expel hydrochloric acid. Finally dilute, cool and filter on a soft paper whose approximate weight is known. If the filtrate is not perfectly clear, refilter. Wash the paper free from any platinum stain and if any appreciable residue remains on the paper, dry and weigh it on the filter. Correct the weight of platinum for this weight of carbon, or other residue, then make the solution to the desired concentration. For potassium determinations the solution should contain 10 per cent of the element platinum.

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[1] Delong, *Chem. Weekblad*, **10**, 833 (1914).

Perchlorate Method.—Potassium perchlorate is nearly insoluble in 97-per cent alcohol while sodium perchlorate is quite soluble. Potassium may be precipitated and separated from sodium by making use of this difference in solubility. A 60-per cent solution of perchloric acid is generally used. This solution does not deteriorate on standing and it is not dangerous to handle, as is the pure acid. It is necessary to have the solution free from ammonium salts since ammonium perchlorate is only slightly soluble in alcohol.

Determination of Potassium: *Perchlorate Method.*[1]—The solution of potassium and sodium salts, obtained by the Smith method (page 244) is used for this determination. Evaporate to about 25 cc and add 1 to 2 cc of 60-per cent perchloric acid solution. Evaporate in a hood until white fumes of perchloric acid appear, cool and dissolve the residue in a small amount of hot water. Again add 1 cc of perchloric acid solution and evaporate until the solution evolves dense white fumes of perchloric acid. Cool to room temperature and add 25 cc of a solution made by mixing 1 cc of 60-per cent perchloric acid with 300 cc of 97 to 98-per cent alcohol. If the insoluble potassium perchlorate is caked it should be broken with a stirring rod so that no soluble salts will escape the action of the alcohol.

During the process of evaporation of the various solutions a Gooch filter should be prepared, the asbestos felt being washed with the perchloric acid-alcohol mixture. The filter is dried for one hour at 120° to 130°, cooled and weighed. Filter the solution on this prepared filter, removing every trace of the precipitate from the beaker by means of a policeman and the prepared washing solution, and wash four or five times with this solution. Dry for one hour at 120° to 130°, cool and weigh.

From the weight of potassium perchlorate thus obtained calculate the per cent of potassium in the sample.

Loss on Ignition.—Loss due to igniting the soil in contact with air includes that due to the volatilization of ammonium salts and water of hydration, to combustion of organic matter, and to decomposition of carbonates and sulphides. This loss may be reduced, in some instances, by the oxidation of ferrous iron.

Determination of Loss on Ignition.—The samples of dry soil obtained in the moisture determination are heated slowly to redness in a muffle furnace, using the same crucibles, until the organic matter is destroyed. The crucibles are then cooled in a desiccator and weighed and the per cent of loss is calculated.

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[1] Scholl, *J. Am. Chem. Soc.*, **36**, 2085 (1914).

Organic Matter.—In a natural soil there is a close relationship between the proportion of organic matter and the fertility. The cause of this is partly physical (improving the texture of a soil increases its absorbing capacity) and partly biological in that promoting the growth of bacteria, molds and protozoa helps to release essential elements to further availability. Organic matter also furnishes plant food directly. Many definite chemical compounds have been isolated[1] from the complex soil organic materials.

Methods for Determining Total Organic Matter.—An approximate calculation of organic matter may be made from the per cent of carbon, the average carbon of soil organic matter being taken as 58 per cent.[2]

Loss on ignition, as already determined, is sometimes taken as an approximate measure of organic matter. The results obtained by this method usually differ considerably from those obtained by calculating the organic matter from carbon, for reasons already explained.

Of the various methods that have been used for the determination of carbon, direct combustion and oxidation by a mixture of chromic and sulphuric acids have been most widely adopted. At present, due chiefly to the efficiency of the modern electric furnace and to failure to obtain complete oxidation by other methods, the direct combustion method has found greatest favor. By any of these methods, carbon dioxide of carbonates is measured along with that produced by the oxidation of organic carbon and this occasions an error in organic matter calculations, unless carbonate carbon is determined and a correction applied.

The combustion method is similar to that used for the determination of carbon in iron and steel. It depends upon the direct combustion of the soil in a current of oxygen, the carbon dioxide produced being absorbed in standard barium hydroxide and the excess of base titrated.

Warrington and Peak illustrate the discrepancies between the results obtained by calculating organic matter from loss on ignition and from carbon determinations by the following table:

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[1] *U. S. Dept. of Agr., Soils, Bull.* **74** (1910).

[2] See also READ AND RIDDGELL, *Soil Sci.*, **13,** 1 (1922).

TABLE XXII.—ORGANIC MATTER BY TWO METHODS

Kind of soil	Per cent loss on ignition after drying at			Organic matter calculated from carbon
	100°	120°	150°	
Old pasture	9.27	9.06	8.50	6.12
New pasture	7.07	6.88	6.55	4.16
Arable soil	5.95	5.70	5.61	2.44
Clay subsoil	....	5.39	4.76	0.65

Carbonate Carbon.—Carbon dioxide of carbonates varies from 0.03 to 0.25 per cent in all but limestone soils. It is necessary to know the amount of carbonate carbon in a soil before that present in organic form can be calculated.

The method for the determination of carbonate carbon depends upon the decomposition of the carbonate with dilute hydrochloric acid and the passing of the gas into standard barium hydroxide, the excess of base being titrated with standard acid. See page 82, Part I, for details of the method.

Determination of Total Carbon.—The apparatus (Fig. 57) consists of the following parts: A steel cylinder, *A*, containing oxygen under pressure; a bottle, *B*, containing 30-per cent potassium hydroxide solution followed by

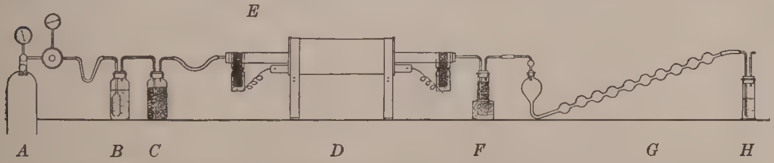

FIG. 57.—Apparatus for the determination of carbon by combustion.

C, containing soda lime to remove possible traces of carbon dioxide from the oxygen. *D*, an electric tube furnace 30 cm long fitted with a combustion tube, *E*, of fused quartz, vitreous silica or porcelain, 60 cm long and with an inside diameter of 2.5 cm, to serve for the combustion. To insure complete oxidation of carbon monoxide, the last half of the portion of the combustion tube which is inside the furnace is filled loosely with platinized asbestos, which acts as a catalyzer.

Connection with the combustion tube is made by means of one-hole rubber stoppers and short glass tubes. The ends of the combustion tube, containing the rubber stoppers, are cooled by means of cotton wicks which dip into bottles containing *distilled* water. (Ordinary ground water will deposit a crust of salts in the wick, this finally stopping capillary action.)

A small bottle, *F*, containing granulated zinc, is attached to the combustion tube. This absorbs chlorine and oxides of sulphur from the products of combustion. Connected with this bottle is the set of Meyer absorption bulbs, *G*, containing standard barium hydroxide. The tube *H* contains soda lime and this protects the barium hydroxide from the carbon dioxide of the air.

The furnace should be heated to about 950° (bright red) and the stopcock opened so as to permit oxygen to pass through at the rate of about 1000 cc in 20 minutes.

Prepare solutions as follows:

(*a*) *Barium Hydroxide.*—A saturated solution of the base is first made by warming and stirring the solid with recently boiled water, using a ratio of 70 to 100 gm of the base to 1000 cc of water, according to the purity of the barium hydroxide obtainable. Cool to room temperature and siphon into a bottle, which is then closed with a rubber stopper. Dilute 550 cc of this solution to 1000 cc with recently boiled and cooled distilled water, mix and place in a bottle which is provided with a guard tube of soda lime and a siphon or similar outlet. (For a method for protecting this and the other solutions, see Fig. 22, page 84.)

(*b*) *Hydrochloric Acid.*—Calculate the dilution such that 1 cc shall be equivalent to 0.002 gm of carbon and make the solution from recently boiled and cooled distilled water. Standardize against sodium carbonate, using methyl orange. Refer to page 82, Part I.

(*c*) *Water, Free from Carbon Dioxide.*—Boil distilled water for 5 minutes and then cool rapidly and preserve in bottles provided with siphon outlets and soda lime guard tubes. Instead of boiling, a current of air may be drawn through the water (best slightly warmed) for one hour, the air first passing through soda lime. *This water is not to be used in an ordinary wash bottle, from which water is expelled by blowing.*

Blanks.—Rinse the Meyer bulbs with water (*c*), then measure into them from a burette or an automatic pipette attached to the bottle, 50 cc of the dilute barium hydroxide solution, first discarding the few drops that are in the outlet of the measuring instrument. Add to the bulbs from a graduated cylinder enough water (*c*) to bring the liquid to the lower edge of the upper bulb when the gas is flowing. The quantity necessary should be determined, once for all, so that it may be added without delay in subsequent determinations. With the furnace already heated, connect the bulbs in place while the oxygen is flowing at the rate of about three bubbles per second. At the end of 15 minutes, disconnect the bulbs without stopping the flow of gas and replace with a second set of bulbs, similarly charged.

Rinse the barium hydroxide solution from the first set into a 500-cc Erlenmeyer flask, using water (*c*). Pay no attention to any precipitate that may remain in the bulbs. Add a drop of phenolphthalein and titrate at once with the standard hydrochloric acid. The acid must not be added too rapidly and the solution must be stirred continuously, so that no local excess of acid may be attained. Note the volume of acid required to discharge the color. The pink color may return as the solution is allowed to stand but this is not considered in the reading.

At the end of 15 minutes from the time the second set of bulbs was inserted, replace the first bulbs, recharged with barium hydroxide. The titration of the second solution constitutes the second "blank" and the average of this and the first is to be taken as the acid equivalent of the barium hydroxide solution.

While one or more blank determinations are running weigh 2 gm of 100-mesh soil and transfer it to an alundum boat (about 10 cm long and as wide as the tube will allow) and mix the soil with an equal weight of 20-mesh alundum. Replace the Meyer absorption tube by another, containing exactly 50 cc of fifth-normal barium hydroxide solution. Place the boat in the combustion tube, connect and continue to pass oxygen for 20 minutes. At the end of this period, disconnect the absorption tube and replace by a second, containing barium hydroxide as before. Without interfering with the flow of oxygen, immediately withdraw the boat from the tube and insert another, containing a sample weight as before. Insert the stopper carrying the oxygen tube and while combustion is proceeding with the second sample, rinse the barium hydroxide from the first Meyer tube into a 500-cc Erlenmeyer flask, using 50 cc of carbon dioxide-free water, and titrate the unused excess with standard acid, using phenolphthalein as indicator. Calculate the per cent of total carbon and from this the per cent of organic carbon, deducting that present in the carbonate form.

Soil Humus.—This is a somewhat indefinite term, used to designate an intermediate stage of decomposition of the complex organic residues usually found in the soil. The term "humus" is arbitrarily used to include that part of the soil organic matter which has reached a stage of decomposition in which it is soluble in 4-per cent ammonium hydroxide. Part of this decomposed organic matter contains certain substances having acid properties, which combine with basic materials to form organic salts called humates. *Total humus material* is the active available organic plant food, while the residual organic matter is useful in improving the soil texture.

There has been considerable discussion concerning the real value of the humus determination. While it must be admitted that the term "humus" does not cover a sharply defined class of compounds and that the result of the determination is subject to considerable variation unless the method is rigidly standardized, it yet appears that some useful information is obtained, in at least approximately classifying organic matter into easily and immediately available forms and those not so available.[1] The determination is no longer official.

Determination of Humus.—Five-gram samples of air-dried soil, ground to pass a 60-mesh sieve, are placed in 500-cc wide-mouthed bottles and washed repeatedly by shaking with a 1-per cent solution of hydrochloric acid until calcium and magnesium are no longer extracted, as shown by testing a small quantity of filtered solution with ammonium hydroxide and ammonium oxalate. The first washings need not be tested. The washing can be hurried by manipulating the bottle in a shaking machine for 15 minutes (Fig. 51). After calcium and magnesium have been removed, filter the solution and wash the soil free from acid by decantation. Return the filter and its contents to the bottle and add 250 cc of 4-per cent ammonium hydroxide. Shake in the machine for three hours, or every 30 minutes by hand for six hours, then place the bottle in a horizontal position for twelve hours.

Again shake the bottle well and pour the contents upon a 24-cm filter paper in a funnel. Cover the funnel with a watch glass. The filtrate may be very turbid for an hour or more. In this case, refilter. When the filtrate comes through clear, save 100 cc or more of it in a clean flask. Pipette 50 cc of the clear filtrate into an 8-cm evaporating dish. Evaporate to dryness on a steam bath, dry in the oven for an hour at 100°, cool in a desiccator and weigh. Burn the carbonaceous matter to an ash in the muffle furnace, cool, weigh and calculate the difference between the two weights as per cent of humus.

Extraction of Material Soluble in Strong Acid.—As in the case of organic matter, the inorganic constituents of the soil are combined in forms which differ widely in degree of availability. Calcium may be present either as limestone (calcium carbonate) which is easily soluble in acids, or as one or more of a variety of silicates, such as anorthite (calcium aluminium silicate) which is nearly insoluble. A similar variation exists with potassium, which may be present as a soluble carbonate or as orthoclase, a silicate of potassium and aluminium which is highly insoluble. Extraction of the soil with hydrochloric acid provides an approximate distinction between materials of small availability and the more available ones. The acid extract may be evaporated to dryness and the extract simply weighed, or the residue may be subjected to a partial or complete analysis as outlined for the original soil.

The amount of material dissolved by the acid varies with the concentration of the latter, the fineness of division of the soil particles and the length of heating. It is therefore obvious that such an extraction constitutes only a conventional division into somewhat arbitrary classes of materials.

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[1] *Soil Science*, **3**, 515 (1917).

Other Inorganic Constituents.—The methods outlined in the following pages may be applied to the analysis of the original soil or of an acid extract, as explained above. As the soil always contains a large proportion of materials insoluble in acids the principal analysis must be preceded by a decomposition such as will bring the sample into complete solution, with the exception of silica, which is separated and weighed. Two methods for the decomposition will be discussed.

Decomposition of Soil Sample: (*a*) *Hydrofluoric Acid Method.*—The soil is treated with hydrofluoric acid and a small amount of sulphuric acid. Silica of silicates is volatilized as silicon tetrafluoride:

$$SiO_2 + 4HF \rightarrow SiF_4 + 2H_2O.$$

Metals are left as sulphates, which decompose by ignition, leaving oxides. These are, for the most part, soluble in hydrochloric acid. This method of soil decomposition is often used where a determination of silica is not required. It obviates, to some extent, the difficulties which are encountered when silica is determined in the same sample with the other constituents but the residue of oxides is often difficult to dissolve and the fusion method is to be preferred.

(*b*) *Fusion with Alkali Carbonates.*—The decomposition of a soil by fusing with sodium carbonate is of greatest use when total silica is to be determined. The silica is usually chiefly combined with sodium and aluminium in albite ($NaAlSi_3O_8$), with potassium and aluminium in orthoclase ($KAlSi_3O_8$), or with aluminium in clay ($Al_2Si_2O_7 \cdot nH_2O$). When orthoclase, for example, is fused with sodium carbonate, the reaction which takes place may be represented as follows:

$$2KAlSi_3O_8 + 6Na_2CO_3 \rightarrow K_2SiO_3 + 5Na_2SiO_3 + 2NaAlO_2 + 6CO_2. \quad (1)$$

The silicates formed in the above reaction may be decomposed easily by hydrochloric acid, forming soluble alkali chlorides and silicic acid:

$$Na_2SiO_3 + 2HCl \rightarrow H_2SiO_3 + 2NaCl. \quad (2)$$

There are also formed soluble chlorides of iron, aluminium, calcium and such other metals as were present in the soil.

The silica is separated almost completely from the other compounds by evaporation to dryness and heating to about 120° to decompose the silicic acid:

$$H_2SiO_3 \rightarrow H_2O + SiO_2. \tag{3}$$

The residue is taken up with water and hydrochloric acid and the insoluble silica is separated by filtration. However, this separation is incomplete as there is a tendency to form a hydrosol of silicic acid. The error thus produced may be avoided by filtering off the silica formed on first evaporation and repeating the dehydration of soluble silica by a second evaporation. The silica finally obtained is not pure but the amount of impurities may be determined by treating the ignited and weighed precipitate with hydrofluoric acid, thus converting the silica into silicon tetrafluoride. The latter is volatilized by heating, leaving oxides of iron and aluminium as a residue.

Silica.—The function of silicon in plant growth is not well understood. There is a considerable amount of this element in some plants (notably oat and rye straw) and it may serve some useful purpose, not yet understood.

Aluminium.—Compounds of aluminium are present in normal soils in rather large quantities. The per cent of aluminium in sandy loam is about 1.5, in clay loam, 4.5, and in residual soils formed from gneiss or limestone about 13. Residual soils usually contain much more aluminium and iron than do glacial soils. Salts containing aluminium are present in some acid soils in sufficient amount to exert a toxic influence on certain plants; barley and corn are particularly sensitive to it. This effect is probably due to the existence of colloidal basic aluminium salts which are capable of being absorbed by the plant. The toxicity may be corrected to a considerable extent by an application of calcium silicate, acid phosphate, or limestone to the soil, thus causing the aluminium to form a less soluble compound.

Iron.—The iron content of soils is quite variable. In soils only slightly tinted, from 1.5 to 4 per cent of iron, calculated as ferric oxide, is found. Ferruginous loams contain from 3.5 to 7 per cent and the red lands from 7 to 14 per cent.

Iron and aluminium are precipitated together as hydroxides. If titanium is present in the soil the precipitate will contain also titanium hydroxide. Phosphorus will be precipitated here as basic ferric phosphate. The combined precipitate is ignited and the oxides and phosphate weighed together. Iron is then determined by dissolving and titrating with a standard permanganate or dichromate solution. Phosphorus is determined in a separate sample and calculated to the pentoxide, while titanium is usually ignored unless it is known to be present in appreciable quantities, as it has no known biological significance. The sum of the per cents of oxides of iron and phosphorus, subtracted from the per cent of total residue, gives the per cent of impure aluminium oxide.

Direct Method for Determining Aluminium.—The above procedure necessarily throws the combined errors of all of these determinations upon aluminium. If an accurate determination of the latter is required, a direct determination may be made. In this case the precipitate of hydroxides is redissolved without previous ignition and the iron is reduced to the ferrous condition by sodium thiosulphate:

$$2Na_2S_2O_3 + 2FeCl_3 \rightarrow Na_2S_4O_6 + 2FeCl_2 + 2NaCl.$$

The aluminium is then precipitated as phosphate, ferrous phosphate remaining in solution.

Purification by Double Precipitation.—The precipitates of iron and aluminium hydroxides, of calcium oxalate and of magnesium ammonium phosphate are difficult to purify by simple washing. If accuracy is important, purification is usually accomplished by dissolving the partly washed precipitate, redissolving and reprecipitating. In the solution from which the second precipitation is made the concentration of soluble salts is only a small fraction of that in the original solution and the amount now carried down by the precipitate and not removed by washing is extremely small.

Calcium.—Many soils that are noted for their fertility have a high calcium carbonate content. Examples of such are the blue grass soils of Kentucky, the calcareous prairie soils of Illinois and Indiana and the black prairie soils of Texas and Mississippi.

Calcium functions particularly in stimulating root development and it is thought to be connected in some way with the development of cell wall material. Some crops, such as alfalfa, clover, and tobacco, require large amounts of calcium for good growth and development.

For the determination, calcium is precipitated from the filtrate from iron and aluminium as calcium oxalate. The calcium may then be determined gravimetrically, as oxide, or volumetrically by titration with standard potassium permanganate. These determinations are discussed on pages 63 to 69, Part I.

Magnesium.—This is a plant food element which plays an important part in seed production as magnesium, like phosphorus, moves to the seed to a great extent. In this respect it is unlike potassium and calcium, which remain largely in the stem and leaf. Magnesium appears also to function in oil and chlorophyll production.

Magnesium is determined in the filtrate from calcium oxalate by precipitating as magnesium ammonium phosphate in a solution previously made basic with ammonium hydroxide. The precipitate is ignited and weighed as magnesium pyrophosphate. The principles underlying this determination have been discussed in connection with the analysis of phosphate, page 87, Part I. When magnesium is being determined, a soluble phosphate is used as the reagent.

Determination of Total Silica.—Weigh accurately about 1 gm of soil into a platinum crucible, burn off the organic matter and when cool mix with approximately 10 gm of sodium carbonate. Place the cover on the crucible slightly to one side so that the contents may be observed. Heat gently at first, using a small burner. Gradually raise the temperature to that of the full flame and heat until gas evolution is only slight. Place the crucible over a blast lamp and heat for at least 15 minutes after the evolution of carbon dioxide has ceased. While it is still hot, rotate the crucible by manipulating the triangle, so that the fused mass will spread over the sides as it solidifies. When it has cooled, place the crucible on its side in a casserole and cover with hot distilled water.

Heat until the fused mass has disintegrated, cover and gradually add 15 cc of concentrated hydrochloric acid from a pipette through the lip of the casserole. Place on a steam bath and, after all effervescence has ceased, remove the crucible and cover, rinsing well. Use a stirring rod for this purpose. By inserting this in the mouth of the crucible the latter can be raised out of the solution and the outside thoroughly rinsed. It can then be taken in the hand and the interior rinsed. A policeman may have to be used if silicic acid adheres to the crucible. *Do not use metal crucible tongs for removing crucibles from solutions, especially if the latter are acid, as in this case.*

Evaporate the liquid to dryness over a steam bath, or keep the casserole in constant motion over a low flame. Heat for 15 minutes at 120° in an oven constructed of material that will not be damaged by acid vapors, or to just below redness over a flame. When cool, add 5 cc of concentrated hydrochloric acid and 75 cc of water, heat until soluble salts are dissolved, filter off the silica and wash the paper and silica with hot water until free from acid. Repeat the evaporation of the filtrate and washings and treat as before, using a different filter paper. Save the filtrates and washings for the determination of other inorganic constituents.

Burn both filter papers in one platinum crucible (which need not be weighed previously) then ignite over a blast lamp, cool and weigh. Add a few drops of sulphuric acid and about 5 cc of hydrofluoric acid (pouring the latter directly from the bottle) to the material in the platinum crucible and volatilize the silicon tetrafluoride and acids by evaporation to dryness under a hood. Ignite the residue and weigh. The loss in weight represents silica. Calculate the per cent.

The residue in the crucible consists of oxides of iron and aluminium. Add about 1 gm of potassium pyrosulphate and heat, gradually raising the temperature to redness, until solution of the oxides is complete. Cool and dissolve the fusion in hot water. Precipitate the metals as hydroxides, as directed below, wash and discard the filtrate and washings. Preserve the precipitate on the paper, so that it may be burned in the same crucible as the main precipitate of iron and aluminium hydroxides, the total oxides being weighed together.

Determination of Iron and Aluminium: *Direct Method for Iron, Indirect Method for Aluminium.*—Dilute the filtrate from the determination of silica to about 75 cc. Add a drop of methyl red and then add dilute ammonium hydroxide until the solution is distinctly basic, avoiding an undue excess. Boil for 5 minutes or until the odor of ammonia is faint, but without prolonging the boiling until the solution becomes acid in reaction. Filter the precipitate through an extracted paper and wash with hot water two or three times. Return the precipitate to the first beaker and dissolve in warm water containing a small amount of hydrochloric acid. Reprecipitate, filter and wash the precipitate free from chlorides. Save the filtrate and washings from both precipitations for the determination of calcium and magnesium.

Burn the paper at a low temperature in a weighed platinum crucible, inclining the crucible to facilitate oxidation. When most of the carbon has been removed, add the paper containing the iron and aluminium hydroxides from the silica determination (see above) and burn this in the same manner. Finally heat to bright redness, cool in a desiccator and weigh as oxides of iron (ferric), aluminium, titanium and phosphorus.

Unless the residue of oxides is white, add about 2 gm of potassium pyrosulphate and heat, gradually raising the temperature to bright redness.

After solution appears to be complete, cool and place the crucible on its side in 50 cc of hot water in a beaker or casserole. Warm to hasten solution of the mass of sulphates. Remove and rinse the crucible, heat to boiling and add 1 cc of 5-per cent stannous chloride solution to reduce the iron. Cool rapidly in running water and add, *all at once*, 50 cc of 5-per cent mercuric chloride solution. This must produce a precipitate of pure white mercurous chloride. If no precipitate is produced, not enough stannous chloride was added. If the precipitate is gray, instead of white, too much stannous chloride was used.

Titrate at once with standard potassium dichromate, following the details of the method described on page 74, Part I. Or the determination may be made with standard potassium permanganate as directed on page 72, Part I. Calculate the per cent of the total oxide and phosphate residue and of ferric oxide in the soil sample. The latter, together with the per cent of phosphorus pentoxide (as determined in a separate sample), subtracted from the per cent of total residue, gives the per cent of aluminium oxide and titanium oxide. The titanium is usually ignored, as already stated.

Determination of Aluminium: *Direct Method.*—Make the double precipitation of hydroxides and wash free from chlorides, as directed above, saving the filtrates and washings for the determination of calcium and magnesium.

Place a 500-cc beaker under the filter and redissolve the precipitate with warm dilute hydrochloric acid. Pierce the filter and wash the paper well with hot water. Dilute the solution to about 400 cc. Add 30 cc of a 10-per cent solution of ammonium phosphate and then stir and add dilute ammonium hydroxide until a precipitate appears. Add 1.5 cc of concentrated hydrochloric acid and 50 cc of a 20-per cent solution of sodium thiosulphate and boil for a few minutes. Now add 15 cc of a 20-per cent solution of ammonium acetate and 8 cc of 30-per cent acetic acid and boil for 15 minutes. The colloidal aluminium phosphate becomes granular and it is then easily filtered and washed. Save the filtrate and washings for the iron determination unless the original precipitate of hydroxides was perfectly white, indicating the presence of no more than a trace of iron.

Redissolve the phosphate on the filter with concentrated hydrochloric acid, wash through with hot water and reprecipitate aluminium phosphate exactly as before. Wash with hot water, ignite and weigh as aluminium phosphate, $AlPO_4$. Calculate the per cent of aluminium oxide in the sample.

Determination of Calcium.—Evaporate the combined filtrates from aluminium and iron hydroxides to about 50 cc, cool, add ammonium sulphide to precipitate the manganese, filter and wash with hot water. Discard the precipitate. Again evaporate the solution to about 50 cc, make slightly basic with ammonium hydroxide and add, while still hot, 4-per cent ammonium oxalate solution, dropwise and with stirring, so long as any precipitate is produced. Heat to boiling, allow to stand one hour or longer, decant the clear solution on a filter, pour about 20 cc of hot water on the precipitate and again decant the clear solution on the filter. Dissolve the precipitate in the beaker with a few drops of hydrochloric acid, add 15 cc of water and reprecipitate by adding ammonium hydroxide and ammonium oxalate solution as before. Allow to stand for an hour and filter through the same paper. Wash the beaker and precipitate with hot water until free from chlorides. Save the filtrate and washings from both precipitations for the determination of magnesium.

Determine the calcium either gravimetrically or volumetrically.

(*a*) *Gravimetric Method.*—Place the paper and calcium oxalate in a weighed crucible, heat carefully until dry and then ignite in the covered crucible for 30 minutes over a blast lamp or a Méker burner. Weigh as calcium oxide and calculate the per cent of this in the sample.

(*b*) *Volumetric Method.*—Dissolve the calcium oxalate and titrate with potassium permanganate, following the details outlined on page 69, Part I. Calculate the per cent of calcium oxide in the soil sample.

Determination of Magnesium.—Acidify the filtrate from calcium with hydrochloric acid and evaporate until ammonium chloride or oxalate begins to crystallize. Add 10 cc of water and stir until the salts are in solution. To the filtrate add a drop of methyl red and sufficient ammonium hydroxide to make the solution barely basic. Now add from a pipette, slowly and with stirring, 20 cc of a 10-per cent solution of disodium orthophosphate. Let stand for 20 minutes or until crystallization begins, then stir and add a quantity of concentrated ammonium hydroxide about equal in volume to one-ninth of the total. Cover the beaker and let stand for three hours or over night. Filter on paper, making no effort to remove adhering precipitate from the beaker. Wash two or three times with dilute ammonium hydroxide and discard the filtrate and washings. Dissolve the precipitate on the filter with hydrochloric acid and allow the solution to run into the beaker containing some of the precipitate. Wash down the paper thoroughly with hot water, dilute to about 75 cc and precipitate the magnesium as before. Filter the precipitate in an ignited and weighed alundum crucible and wash until free from chlorides with a 2-per cent solution of ammonium hydroxide, testing the washings finally with silver nitrate solution made acid with nitric acid. Cover the crucible and heat gently over a burner until dry and finally heat for 20 minutes, using a blast lamp. Cool in the desiccator and weigh. From the weight of magnesium pyrophosphate calculate the per cent of magnesium in the sample.

Manganese.—Manganese is present to some extent in alluvial clay soils but it is more abundant in volcanic clays. In small amounts, approximating not more than about 50 lb. of manganese per acre of soil, $6\frac{2}{3}$ in. deep (0.0025 per cent), it seems to have a stimulating effect on plant growth. Many plant compounds contain manganese but its biological function is not well understood.

The manganese content of a large number of different legumes (aerial portion) was determined by Jones and Bullis,[1] who found alsike clover to have the greatest amount, averaging 0.068 per cent, while alfalfa had the least, with 0.023 per cent.

Work on the effect of manganese has been done also by Kelley,[2] who concluded that manganese is a plant food, when present in small amounts, but that in larger quantities it becomes toxic.

In some Hawaiian soils the per cent of manganese is so high as to interfere with the growth of the pineapple, causing a depression in iron assimilation.[3]

The bismuthate method for the determination of manganese is one of the best. It is based upon the use of sodium bismuthate to oxidize bivalent manganese to heptavalent manganese in the form of permanganic acid. When a solution of manganous nitrate is treated with sodium bismuthate the reaction proceeds thus:

$$2Mn(NO_3)_2 + 5NaBiO_3 + 14HNO_3 \rightarrow 2NaMnO_4 + 3NaNO_3 + 5Bi(NO_3)_3 + 7H_2O.$$

Sodium permanganate so produced is reduced by means of a standard reducing agent, the excess of which is then titrated with standard permanganate solution.

Persulphate Method for Manganese.—Manganese may be oxidized by ammonium persulphate, in the presence of silver nitrate, from a bivalent to a heptavalent condition, producing permanganic acid:

$$(NH_4)_2S_2O_8 + 2AgNO_3 \rightarrow Ag_2S_2O_8 + 2NH_4NO_3, \quad (1)$$

$$Ag_2S_2O_8 + 2H_2O \rightarrow 2H_2SO_4 + Ag_2O_2, \quad (2)$$

$$5Ag_2O_2 + 2Mn(NO_3)_2 + 6HNO_3 \rightarrow 2HMnO_4 + 10AgNO_3 + 2H_2O. \quad (3)$$

The manganese is determined in an extract from the soil fusion by comparing the intensity of color produced in this manner with that of a manganese solution of known concentration, similarly treated, or by titration with a standard reducing agent such as ferrous ammonium sulphate or sodium arsenite:

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[1] *J. Ind. Eng. Chem.*, **13**, 6 (1921). See also McHargue, *J. Am. Chem. Soc.*, **44**, 1592 (1922).

[2] *Hawaii Exp. Sta. Bull.* **26** (1912).

[3] Johnson, *Ibid.*, **9**, 1 (1917).

$$2HMnO_4 + 5Na_3AsO_3 + 4HNO_3 \rightarrow 5Na_3AsO_4 + 2Mn(NO_3)_2 + 3H_2O.$$

Determination of Manganese: *Bismuthate Method.*—Prepare the following solutions:

(*a*) *Potassium permanganate solution,* 1 cc of which is equivalent to 0.0001 gm of manganese by the following reaction:

$$KMnO_4 + 5Fe(NO_3)_2 + 8HNO_3 \rightarrow KNO_3 + Mn(NO_3)_2 + 5Fe(NO_3)_3 + 4H_2O.$$

Standardize against ferrous ammonium sulphate or sodium oxalate, as directed on page 68, Part I.

(*b*) *Ferrous ammonium sulphate solution,* the concentration to be about equivalent to that of the potassium permanganate solution and containing 50 cc of concentrated sulphuric acid in each 1000 cc. The solution is standardized by blank titrations at the time it is used in the determination of manganese.

(*c*) *Nitric Acid, Specific Gravity* 1.13.—Dilute one volume of the concentrated acid with three volumes of water.

(*d*) *Nitric Acid, Specific Gravity* 1.015.—Dilute three volumes of the concentrated acid with 100 volumes of water.

Ignite 1 gm of soil gently in air until all organic matter is burned. Fuse the ignited soil as directed for the silicon determination, page 256. After the fusion is perfectly fluid place the cooled crucible on its side in a covered casserole and add nitric acid (*c*) until the sodium carbonate is decomposed. Rinse and remove the crucible and evaporate the solution to dryness on the steam bath. Finally take up with 50 cc of nitric acid (*c*). Heat to aid in solution but do not evaporate much of the acid. Cool, add about 0.5 gm of sodium bismuthate and stir. After 10 minutes add 50 cc of nitric acid (*d*) and filter the whole through a Gooch filter, using suction. After filtering, wash the beaker and crucible with 50 cc of the same nitric acid. From a burette add to the filtered solution 35 cc (more if necessary to reduce all permanganate) of ferrous ammonium sulphate solution (*b*). The permanganate is reduced and there is an excess of ferrous salt present. Titrate this excess to a faint pink color with standard potassium permanganate solution (*a*).

A blank determination is made, using 50 cc of dilute nitric acid, 50 cc of nitric acid (*d*) and 0.25 gm of sodium bismuthate. Filter through asbestos and wash with 50 cc of nitric acid as in the previous determination. From a burette add 35 cc of ferrous ammonium sulphate solution and immediately titrate with the standard potassium permanganate. The difference between the volumes of permanganate required for the blank and the manganese determination, respectively, is that equivalent to manganese in the sample of soil. Calculate the per cent of manganese.

Determination of Manganese: *Persulphate Method.*—The standard permanganate solution prepared as for the bismuthate method is used in this case.

One gram of soil is fused and treated as directed above for the bismuthate method. Do not add sodium bismuthate but after the residue from evaporation has been dissolved in nitric acid, add 15 cc of a 0.2-per cent silver nitrate solution, following immediately by 1 gm of ammonium persulphate. Heat by placing the beaker or casserole in hot water until the pink color is fully developed. Cool and rinse into a tube of a color comparator. Place in another tube enough of the standard permanganate solution (measured accurately) to make a somewhat greater intensity of color, when viewed from above, dilute to the mark and mix. Place both tubes in the comparator (Fig. 52, page 237) and adjust to equality of color. Calculate the per cent of manganese in the sample.

Sulphur.—The sulphur content of most soils is usually less than that of phosphorus, while considerable sulphur is needed by certain plants to produce proteins and flavoring oils. It has been shown that onions, mustard, and cabbage usually respond favorably to the addition of either elementary sulphur or sulphates to the soil. The function of sulphur in the plant metabolism is not well understood.

The determination of sulphur in soil is preceded by fusion with sodium carbonate in the presence of a small amount of an oxidizing agent, the latter in order to convert protein sulphur to the form of sulphates. The sulphate thus formed, together with sulphates originally present as such, is later precipitated and weighed as barium sulphate. The heating should be done with an alcohol burner or in an electrically heated muffle furnace instead of with a gas flame because of danger of absorption of sulphur dioxide from the burning gas (which always contains hydrogen sulphide) by the sodium carbonate.

Determination of Sulphur.—Mix 2 gm of 100-mesh soil with 7 gm of anhydrous sodium carbonate (free from sulphates) and 0.5 gm of potassium nitrate in a platinum crucible. Place the covered crucible in an electrically heated muffle and heat to dull redness until well fused, after which remove the crucible and tip it in such a manner as to cause the contents to solidify on the sides. While it is still hot place the crucible in 75 cc of cold water in a 200-cc beaker (use care). Cover and heat the beaker and contents to boiling. Stir until all lumps of the fused mass have been disintegrated, then filter into a 400-cc beaker and wash the residue until the volume is about 200 cc. Reduce any sodium manganate present by boiling with a few drops of alcohol, add a drop of methyl red and then add hydrochloric acid from a pipette until neutral. Now add 1 cc of approximately normal hydrochloric acid (or an equivalent volume of acid of other normality). Heat to boiling and add, dropwise and with continuous stirring, enough of a 10-per cent solution of barium chloride to precipitate all sulphates. Digest at nearly boiling until the precipitate settles readily. Filter off the precipitated barium sulphate and wash with hot water until free from chlorides. Carefully burn the paper in an inclined crucible, with free access of air, until white but do not allow the crucible to become bright red and do not heat longer than is necessary to burn all carbon. Calculate the per cent of sulphur in the soil, expressing as the element and as sulphur trioxide.

Lime Requirements of Soils.—Lime is added to acid soils for the purpose of neutralizing their excess of acid but it also changes the physical texture of the soil. In addition to these effects, there is a precipitation of iron and aluminium from soluble salts as hydroxides, in this way lessening their toxicity.

Calcium itself is regarded as one of the necessary elements in the plant economy. There is considerable difference with regard to the need of different plants for calcium and also with respect to their ability to draw this element from the less available sources. Alfalfa is an example of a plant that needs much calcium in its metabolic processes but having a rather limited feeding power while, on the other hand, the rye plant needs much less calcium but possesses ample feeding capacity to secure the little it requires.

It is generally considered that many soils possess acidity through the presence of insoluble acid salts of organic and inorganic acids and a number of methods in use for the determination of soil acidity are based upon this assumption. Certain fertilizers have a tendency to cause a soil to become acid. This is especially true of ammonium sulphate. As nitrogen is taken from this salt by the plant, sulphuric acid remains as a residue in the soil. Green manures have been credited also with producing acid soils, acid being formed during fermentation. However, much confusion still exists concerning the true nature of soil acidity and consequently there is no generally accepted method for its determination. The lime calculated to be required to neutralize acidity varies, therefore, according to the method employed for the determination of acidity.

Veitch Method.[1]—In the Veitch method for the determination of soil acidity a measured quantity of lime water solution of known concentration is evaporated to dryness with a definite amount of soil. The mass is then extracted with distilled water, phenolphthalein is added and the solution is concentrated by boiling. If the quantity of calcium hydroxide added was more than sufficient to neutralize soil acids, an indication of this will be given by a pink color from the phenolphthalein. By this method there is probably some error due to a combination of calcium hydroxide with organic matter and possibly with carbon dioxide from the air.

Determination of Lime Requirement of Soil: *Veitch Method.*—Weigh five portions of 10 gm each of the soil into 8-cm porcelain evaporating dishes. Add fiftieth-normal calcium hydroxide solution in such amounts that it will range from 2 cc below to 2 cc above the probable amount of calcium hydroxide needed, making a difference of 1 cc in the volume of calcium hydroxide for each pair of consecutive members of the series. A series extending over 5 to 10 cc of solution may be used as a beginning. Evaporate all to dryness over the steam bath and immediately take up the residues with distilled water and transfer to 300-cc flasks, using 150 cc of water, previously freed from carbon dioxide by boiling for several minutes in an open beaker or dish. Shake well, stopper and let stand over night, then pipette 50 cc of the clear liquid from each flask into Pyrex beakers. Add a drop of phenolphthalein and heat to boiling, continuing the boiling until two-thirds of the liquid has been boiled away. Note in what beakers, if any, the liquid has turned pink. Repeat, using a narrower series whose limits are indicated by the results on the first series. The least volume of calcium hydroxide solution required to cause a pink tint is equivalent to the lime requirement of 10 gm of soil. Calculate the pounds of calcium carbonate needed on the basis of 2,000,000 lb. of soil per acre.

The Truog Method.[2]—If barium chloride and zinc sulphide are added to an acid soil, evolution of hydrogen sulphide takes place:

$$2RCOOH + BaCl_2 \rightarrow (RCOO)_2Ba + 2HCl, \qquad (1)$$

$$ZnS + 2HCl \rightarrow H_2S + ZnCl_2. \qquad (2)$$

This gas coming in contact with lead acetate paper produces a degree of blackening somewhat in proportion to the amount of acid present.

$$H_2S + Pb(C_2H_3O_2)_2 \rightarrow PbS + 2HC_2H_3O_2. \qquad (3)$$

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[1] *J. Am. Chem. Soc.*, **26**, 261 (1904).

[2] *Wis. Exp. Sta. Bull.*, **249** (1915).

Potassium Thiocyanate Method.—When there is a deficiency of calcium or magnesium carbonate in a soil the aluminium and iron present combine with any free acid radicals to form salts of these metals. If an alcohol solution of potassium thiocyanate is added to such a soil the solution will acquire a red color,[1] the intensity of which has been shown to be approximately proportional to the acidity of the soil. Also if to this red solution an alcoholic solution of logwood be added, a blue color will develop, the intensity of which is again proportional to the concentration of both aluminium and acidity in the soil extract.

As an explanation of this color formation, it may be supposed that, in an acid soil, iron and aluminium exist in the form of partly hydrolyzed, largely colloidal salts in equilibrium with the weakly ionized soil acid. These salts are capable of reacting with such salts as potassium thiocyanate, which would not be true of insoluble oxides or silicates, such as would be present in a neutral or basic soil. This might be expressed thus:

$$\mathrm{Fe}A_3 + \mathrm{H_2O} \rightleftarrows \mathrm{FeOH}\cdot A_2 + \mathrm{H}A, \qquad (1)$$

$$\mathrm{FeOH}.A_2 + 3\mathrm{KCNS} + \mathrm{H}A \rightleftarrows \mathrm{Fe(CNS)_3} + 3\mathrm{K}A + \mathrm{H_2O}, \qquad (2)$$

where A represents any acid radical. The ferric thiocyanate thus produced colors the solution somewhat in proportion to the amount of acid which made iron available for this reaction. The addition of a standard solution of a base decomposes the ferric thiocyanate and destroys the color.

It has been noted in making soil acidity determinations by this method that certain soils cause the supernatant liquid to assume a green color, as the red color of the ferric thiocyanate disappears. Analysis has shown that this color is due to the formation of a manganese compound, which is produced after the solution has been made basic. This green color develops if the soil contains as little as 0.008 per cent of soluble manganese. In most cases it has been found that it starts to develop as soon as the red color entirely disappears, but its intensity is increased if 5 cc more of base be added than that required to titrate to the disappearance of red. This red color disappears when P_H equals about 5.5 while manganese does not start to precipitate as hydroxide until

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[1] Comber, *J. Agr. Sci.*, **10**, 420 (1920).

P_H equals about 7.2, this being completed at about 7.9. It is evident that with such a soil a large amount of limestone would have to be applied to precipitate all of the manganese, and in some instances this cost would be prohibitive.

Determination of Soil Acidity: *Potassium Thiocyanate Method.*[1]—Prepare the following reagents:

(*a*) *Potassium Thiocyanate Solution.*—Prepare a 5-per cent solution in 95-per cent ethyl or methyl alcohol. This solution should become slightly pink ($P_H = 5.4$) upon the addition of methyl red. If necessary, add very dilute potassium hydroxide or hydrochloric acid, drop by drop, until this color is obtained with a few drops added to methyl red on a test plate.

(*b*) *Alcoholic Solution of Potassium Hydroxide.*—Prepare a tenth-normal alcoholic solution of potassium hydroxide by dissolving the base in 95-per cent ethyl or methyl alcohol. Titrate against (*c*), using methyl red.

(*c*) *Alcoholic Solution of Hydrochloric Acid.*—Prepare a tenth-normal alcoholic solution of hydrochloric acid by diluting concentrated acid with 100 volumes of 95-per cent ethyl or methyl alcohol. Standardize against sodium carbonate, first dissolving the weighed salt in a small amount of water. See page 83.

Place 50 gm (25 gm of muck) of 10-mesh air-dried soil in a 100-cc glass-stoppered cylinder or in the lower chamber of the specially designed glass tube shown in Fig. 58. Add 30 cc (50 cc for muck) of potassium thiocyanate solution. Stopper the cylinder and agitate for two minutes. Place in an upright position, allow to settle for several minutes and note the color of the supernatant liquid. If the solution is pink or red, add from the upper burette a few tenths of a cubic centimeter at a time (depending upon the color) of tenth-normal alcoholic solution of potassium hydroxide. Shake well after each addition and allow several minutes to settle. Continue the addition until the red or pink color has just disappeared. Let stand fifteen hours and add more base, if necessary, to remove any pink which may have developed. If too much base has been added titrate back to a faint pink color using tenth-normal alcoholic acid. Note the volume of tenth-normal base required and calculate the pounds of calcium carbonate required to correct the acidity of the soil, on a basis of 2,000,000 lb. of ordinary soil or 1,000,000 lb. of muck soil per acre.

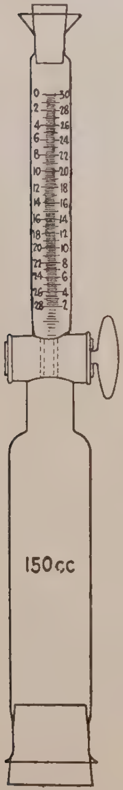

FIG. 58.—Soil acidity burette.

The time required for complete development of color in the thiocyanate solution may be shortened by use of the mixing machine.

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[1] CARR, *J. Ind. Eng. Chem.*, **13**, 931 (1921).

If no red color has developed in the extract, the soil is already basic. In this case, add from a burette tenth-normal alcoholic solution of hydrochloric acid until a pink color develops after standing several minutes, agitating after each addition and then allowing the soil to settle. From the volume of acid used calculate the calcium carbonate equivalent of the soil. If there is any indication of a green color developing after the disappearance of red and after standing over night, add 5 cc more of base. If a green color should develop, it would require from 40 to 50 cc (corresponding to 4 to 5 tons of limestone) of base, in addition to that added to remove the red color.

Hopkins Method.—The acids of the soil (existing in equilibrium with partly hydrolyzed salts, as shown in equation (1), page 265) are not easily extracted with water. If a solution of potassium nitrate is added, such a reaction as the following may occur:

$$\mathrm{H}A + \mathrm{KNO_3} \leftrightarrows \mathrm{K}A + \mathrm{HNO_3}$$

Equilibrium is established with the weakly ionized acid predominating but if the solution is removed and replaced by more potassium nitrate solution, the reaction will proceed still farther. By repeating this process several times, a result is finally obtained, approximating complete extraction of the acid. It has been found by working with a number of different soils that the sum of the acid of such a series of extracts is about two and one-half times that of the first extract. In the Hopkins method the assumption is made that the value of the first titration may be multiplied by 2.5 to give total acidity. The method seems to be more reliable with clay and loam than with muck soils.

Determination of Acidity of Soil: *Hopkins Method.*—Place 100 gm of soil and 250 cc of normal potassium nitrate solution in a 400-cc wide mouthed bottle, stopper and shake continuously in a machine (Fig. 51) for three hours, or every half hour for three hours by hand. Allow to stand for fifteen hours. Draw off 125 cc of the clear solution, using a pipette, boil for 10 minutes to expel carbon dioxide, cool and titrate with tenth-normal sodium or potassium hydroxide, using phenolphthalein as indicator. Multiply the figure so obtained by 2.5 and calculate the number of pounds of calcium carbonate required per acre of 2,000,000 lb. of soil.

The titrations of duplicate samples should not differ by more than 0.8 cc for soil samples requiring less than 100 cc of sodium hydroxide.

Active Plant Food.—The amount of nitrogen, phosphorus and potassium that may be made available in a soil during a given year is of interest and importance. Various weak acids, which imitate the action of the plant roots, have been used for extracting available plant food. Dyer[1] has shown that root acidity (expressed as citric acid) varies from 0.34 to 3.4 per cent of the weight of the plant. He found the average acidity of one hundred plants (root and top) to be about equal to that of 1-per cent citric acid and so used this acid for soil extraction. Fifth-normal nitric and oxalic acids are other solutions that have been used for this purpose. Fifth-normal nitric acid has given best results[2] in field tests and this has been quite widely adopted.

The amount of acid capable of being neutralized by materials already present in the soil also is a factor of importance in fertility work. This is estimated by titrating the solution after the extraction has been completed. The amount of acid consumed depends considerably upon whether the soil is calcareous, it being much greater in this case.

Flocculation and Deflocculation of Clay.—When "silt" soil is suspended in water it may be easily flocculated by a calcium salt, such as calcium nitrate. However, if calcium hydroxide is added so that the solution becomes basic, flocculation is more difficult. A clay responds in just the opposite manner, being easily precipitated from suspension by calcium hydroxide.

Determination of Comparative Degree of Flocculation and Deflocculation.—Place about 3 gm (not accurately weighed) of a clay soil in a mortar and add sufficient water to make a thin paste when rubbed, then dilute to one liter and mix. Repeat this process, using a "silt" soil. Pipette 25 cc of each turbid liquid into each of nine test tubes and add each of the following solutions in order to test its power to flocculate or deflocculate clay and silt soils. The solutions should have approximately the concentrations indicated but they need not be accurately standardized.

(1) Use as a control—water and soil suspension only.
(2) 5 cc of tenth-normal sodium chloride.
(3) 5 cc of tenth-normal monosodium phosphate.
(4) 5 cc of tenth-normal sodium hydroxide.
(5) 5 cc of tenth-normal hydrochloric acid.
(6) 5 cc of tenth-normal ammonium sulphate.
(7) 5 cc of tenth-normal monocalcium phosphate.
(8) 10 cc of twentieth-normal calcium hydroxide.
(9) 5 cc of tenth-normal calcium nitrate.

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[1] *J. Chem. Soc.*, **65**, 115 (1894).

[2] *Ohio Exp. Sta. Bull.*, **261** (1913).

After the reagents have all been added, shake each tube ten times and note the time and the order in which the turbidity of the liquid disappears. Repeat the shaking until the time of clearing is established for each compound added. Note what ion or ions appear to be the most effective in causing flocculation of the soil particles in both types of soils.

CHAPTER XIII

FERTILIZERS

Fertilizers, or manures, are those materials which either increase the supply of elements in the soil, needed for the growth of plants, or exert a corrective action in making conditions more favorable for the plant's best development. Farm manures are usually mixtures of the excrement and urine of farm animals with stable litter.

A distinction is sometimes made between materials which furnish plant food directly, such as nitrates, phosphates and potassium and ammonium salts, and indirect fertilizers like calcium carbonate, which neutralize soil acid as well as serve as plant foods. There are also those which furnish plant food and aid in loosening hard clay, as is the case with manures. The direct fertilizers containing nitrogen, phosphorus and potassium furnish the elements that are most frequently lacking in soils.

Availability.—The value of a fertilizer is usually determined by the per cents of the fertilizing elements and by the solubility of the compounds containing these elements in water or soil acids, also by absence of injurious salts, such as those containing boron or aluminium. Solubility is an obvious measure of availability to plants. The most commonly used, easily available water-soluble salts containing nitrogen are sodium nitrate, ammonium sulphate and calcium cyanamid. Materials in which the nitrogen is available more slowly are manures, legumes in green manuring, stubble and dead roots of plants. In these, nitrogenous organic matter is gradually broken down into simpler, soluble compounds, by bacterial action. Examples of nitrogenous materials in which the nitrogen is practically unavailable are hair, hoof, horn and leather. These are rich in nitrogen but they are insoluble and decompose very slowly in the soil. The phosphate fertilizers also present considerable variation in solubility. This subject is discussed more fully on page 275.

It is therefore a matter of great importance to the analyst that he should know the origin of the constituents of a fertilizer because the methods of analysis and the interpretation of results differ according to the nature of the material present.

The composition of some of the more common fertilizers is indicated in the following table:

TABLE XXIII.—APPROXIMATE COMPOSITION OF CERTAIN COMMERCIAL SAMPLES OF FERTILIZERS WITH RESPECT TO THREE ESSENTIAL ELEMENTS

Name of material	Pounds of element per ton of fertilizer		
	Nitrogen	Phosphorus	Potassium
Fresh farm manure	10	2	8
Dried blood	280	...	...
Sodium nitrate (com.)	310	...	...
Ammonium nitrate (com.)	400	...	...
Acid phosphate (com.)	...	125	...
Acidulated bone meal	40	140	...
Steamed bone meal	20	250	...
Raw bone meal	80	180	...
Raw rock phosphate	...	250	...
Basic slag	...	160	...
Potassium sulphate (com.)	...	...	850
Potassium chloride (com.)	...	...	850
Wood ashes	...	10	100

Compatibility.—When artificial manures are to be mixed it is important to know what ones can be combined without loss of fertilizing value. Losses may be caused by reactions that release combined nitrogen, usually in the form of ammonia, or that make a phosphate less available to the plant by producing less soluble compounds.

When an acid phosphate, for example, is mixed with sodium nitrate or calcium nitrate free nitric acid is produced and this may be partly lost:

$$Ca(H_2PO_4)_2 + 4NaNO_3 \rightarrow CaNaPO_4 + Na_3PO_4 + 4HNO_3.$$

TABLE XXIV.—FERTILIZER COMPATIBILITY

Fertilizer	Should not be mixed with	Mixed just before using with
"Superphosphate," $Ca(H_2PO_4)_2$	lime Thomas slag calcium cyanamid sodium nitrate basic calcium nitrate	
Lime	superphosphate ammonium sulphate bone meal barnyard manure guano	kainit potassium salts
Ammonium sulphate	lime calcium cyanamid basic calcium nitrate Thomas slag	
Calcium cyanamid $CaNCN$	ammonium sulphate superphosphate barnyard manure guano	potassium salts sodium nitrate kainit basic calcium nitrate
Potassium salts	Thomas slag	calcium cyanamid lime basic calcium nitrate
Sodium nitrate	calcium superphosphate	calcium cyanamid basic calcium nitrate
Bone meal	lime	
Kainit, $MgSO_4 \cdot KCl \cdot 3H_2O$.	Thomas slag	calcium cyanamid lime basic calcium nitrate
Basic calcium nitrate	ammonium sulphate superphosphate barnyard manure guano	calcium cyanamid potassium salts sodium nitrate kainit
Barnyard manure and guano	lime calcium cyanamid basic calcium nitrate	
Basic slag ("Thomas slag")	kainit potassium salts ammonium sulphate superphosphate	

Any of the above fertilizers may be mixed, at any time, except as noted otherwise.

A similar reaction is brought about when acid phosphates are mixed with kainit or crude potassium chloride. In the latter case hydrochloric acid is formed slowly and the vapor may be detected with blue litmus paper. Ammonium salts should not be mixed with such basic compounds as hydrated lime or basic slag since a loss of nitrogen in the form of ammonia will result:

$$2NH_4NO_3 + Ca(OH)_2 \rightarrow Ca(NO_3)_2 + 2NH_3 + H_2O. \qquad (2)$$

These basic compounds should not be mixed with farm manures, for the same reason.

If hydrated lime, or any other basic compound were mixed with calcium acid phosphate, the nearly insoluble normal phosphate would be produced:

$$Ca(H_2PO_4)_2 + 2Ca(OH)_2 \rightarrow Ca_3(PO_4)_2 + 4H_2O. \qquad (3)$$

In some cases it is not desirable even to mix fertilizers which do not react with each other (*e.g.*, sodium nitrate and basic slag) because the large difference in the densities of the two compounds makes it difficult to secure thorough mixing. The slag, being much heavier, settles to the bottom of the container as a result of agitation in handling.

Table XXIV shows which fertilizers may be mixed without danger of loss of fertilizing value, which ones should not be mixed until just before applying, and which combinations should never be used.

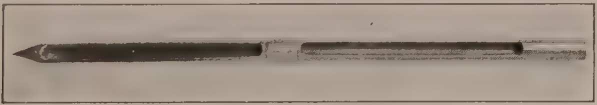

Fig. 59.—Sampler for fertilizers.

Choice and Preparation of a Sample of Commercial Fertilizer for Analysis.—The sample of fertilizer is obtained from the sack or bin by means of a sampler, one form of which is shown in Fig. 59. This should secure a representative portion from the whole mass.

Mechanical Analysis.—Mix the sample well and transfer about 100 gm of it to a sieve having circular openings 0.5 mm in diameter. Break up the soft lumps with a pestle, then sift. Weigh the coarse portion remaining on the sieve. The percentage of the fine portion is determined by difference.

Preparation of Sample.—Refer to the discussion of sampling, pages 17 to 21. Reduce the remainder of the gross sample, by quartering or by use of a riffle, to an amount sufficient for analytical purposes (25 to 50 gm), transfer this to a sieve with 1-mm openings and sift, breaking the lumps with a pestle. Grind the part remaining on the sieve in a mortar until the particles will pass through, mix thoroughly and preserve in tightly stoppered bottles. Carry out these operations as rapidly as possible to avoid loss or gain of moisture during the operation.

Moisture.—Loss of weight on drying may be due to escaped hygroscopic water, chemically combined water or ammonia or, to some extent in certain cases, to oxidation of organic matter. For this reason "moisture" as usually reported, is not a strictly accurate term.

Determination of Moisture.—Weigh 2 gm of the sample into wide crucibles or small dishes and heat for five hours at 100°. In the case of potassium salts, sodium nitrate and ammonium sulphate, heat at about 130° to constant weight. Calculate the loss as percent of moisture.

Phosphorus.—Phosphorus is deficient in soils more often than are the other necessary elements. The mineral phosphates form the chief commercial source of phosphorus, although a considerable amount is obtained from bone, Thomas slag (from the basic Bessemer steel furnace), tankage and fish scrap. Calcium orthophosphate, $Ca_3(PO_4)_2$, is the chief constituent of "raw" rock phosphate. Its solubility in water is very small, in absence of acids, and therefore it is advisable to use it only in a soil where there is considerable decaying organic matter to furnish carbonic acid, as otherwise its availability is small.[1]

Large amounts of rock phosphate are now commercially made into acid phosphates by treating the finely ground stone with sulphuric acid, thus converting the normal phosphate to a soluble form, suitable for use as a fertilizer. The character of the result of this treatment depends upon the concentration of acid and upon the relative amounts of rock phosphate and acid employed in the treatment. Dicalcium or monocalcium phosphate, or even phosphoric acid itself, may be formed, according to whether one, two or three atoms of hydrogen are substituted for calcium. The last two named phosphates are easily soluble in water, whereas dicalcium phosphate is nearly insoluble (0.136 gm in 1000 gm of water at 20°) but soluble in soil acids. In practice the reaction is never allowed to proceed as far as the formation of phosphoric acid.

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[1] See also Hopkins, *Ill. Exp. Sta. Circ.*, **167** (1913) and Stewart, *Ibid.*, **245** (1920).

The possible reactions involved in the commercial process are indicated as follows:

$$Ca_3(PO_4)_2 + H_2SO_4 \rightarrow CaSO_4 + \underset{\text{Dicalcium phosphate}}{2CaHPO_4}, \qquad (1)$$

$$Ca_3(PO_4)_2 + 2H_2SO_4 \rightarrow 2CaSO_4 + \underset{\substack{\text{Monocalcium phosphate} \\ \text{("Superphosphate")}}}{Ca(H_2PO_4)_2}, \qquad (2)$$

$$Ca_3(PO_4)_2 + 3H_2SO_4 \rightarrow 3CaSO_4 + \underset{\text{Phosphoric acid}}{2H_3PO_4}. \qquad (3)$$

Sulphuric acid of 60-per cent concentration is most suitable for making acid phosphates because this produces the maximum quantity of monocalcium phosphate, the water-soluble form. "Reversion" may occur during storage if unchanged tricalcium phosphate remains in the mixture. This is due to the interaction of monocalcium phosphate with tricalcium phosphate, the dicalcium salt being produced:

$$Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \rightarrow 4CaHPO_4.$$

Measure of Availability.—Dicalcium phosphate is soluble in salt solutions, such as ammonium citrate, as well as in salt or acid soil solutions. Hence both citrate-soluble and water-soluble phosphorus are rated as available to plants. The phosphate found in bone is in the form of the tricalcium phosphate but in this case it is in a more porous condition and it is also intermingled with organic matter. It is soluble to the extent of 30 to 40 per cent in ammonium citrate solution and it is somewhat soluble in soil acids and salts.

The principles involved in the determination of phosphorus in phosphates are discussed on pages 87 to 92, Part I. This should be reread before beginning the following determinations.

Determination of Total Phosphorus.—The choice of method for dissolving the sample will depend upon the nature of the latter.

Preparation of Solution.—Treat 2.5 gm of the sample by one of the following methods:

(*a*) Ignite in a crucible until organic matter is removed (the residue will not necessarily be white), then dissolve in hydrochloric acid.

(*b*) Evaporate with 5 cc of magnesium nitrate solution, made as follows: Dissolve 320 gm of calcined magnesium oxide in nitric acid, avoiding an excess of the latter; add a little calcined magnesium oxide in excess, boil, filter from the residue and dilute to 2000 cc.

After evaporating the fertilizer and magnesium nitrate solution, ignite until organic matter is removed and dissolve in hydrochloric acid.

(*c*) Boil with 20 or 30 cc of concentrated sulphuric acid in a Kjeldahl flask, adding 2 to 4 gm of sodium nitrate at the beginning of the digestion and a small quantity after the solution has become nearly colorless, or adding the nitrate in small portions from time to time during the digestion. After the solution is colorless add 150 cc of water and boil for a few minutes.

(*d*) Digest in a Kjeldahl flask with concentrated sulphuric acid and such other reagents as are used in either the plain or modified Kjeldahl or Gunning method for the determination of nitrogen (page 152). Do not add any potassium permanganate but, after the solution has become colorless, add about 100 cc of water and boil for a few minutes.

(*e*) Dissolve in 30 cc of concentrated nitric acid and 5 cc of concentrated hydrochloric acid and boil until organic matter is destroyed.

(*f*) Add 30 cc of concentrated hydrochloric acid, heat and add cautiously, in small quantities at a time, about 0.5 gm of finely pulverized potassium or sodium chlorate to destroy organic matter.

(*g*) Dissolve in 15 to 30 cc of concentrated hydrochloric acid and 3 to 10 cc of concentrated nitric acid. This method is recommended for fertilizers containing much ferric or aluminium phosphate.

After the sample of fertilizer has been brought into solution by any of the methods described above, cool, dilute to 250 cc, mix and pour into a dry filter, discarding the first 10 cc of the filtrate and allowing the remainder to run into a dry flask which can be stoppered.

Gravimetric Determination.—Prepare solutions of "magnesia mixture" and ammonium molybdate as directed on pages 88 and 89, Part I. Prepare also:

(*a*) *Ammonium Hydroxide.*—Dilute the concentrated solution ten times.

(*b*) *Ammonium Nitrate.*—A 10-per cent solution.

Measure 25, 50 or 100 cc of the fertilizer solution, according to the probable per cent of phosphorus, using a pipette or volumetric flask. Transfer to a 250-cc flask of resistance glass, neutralize with ammonium hydroxide and clear with a few drops of nitric acid, thus dissolving the small amount of precipitated hydroxides of iron and aluminium. In case hydrochloric or sulphuric acid has been used as a solvent for the fertilizer material add also 15 gm of dry ammonium nitrate.

To the hot solution add ammonium molybdate solution, about 70 cc for each decigram of phosphorus pentoxide thought to be present. Immerse in water and digest at 65° for an hour and determine whether the phosphorus has been completely precipitated, by adding more molybdate solution to the clear, supernatant liquid. If more precipitate forms continue the digestion, followed by testing as before. Filter on paper and wash with cold water or the ammonium nitrate solution (*b*). During the washing the precipitate that adheres to the flask need not be completely removed bu it must be washed.

Place the flask in which precipitation was made under the filter and dissolve the precipitate on the filter in concentrated ammonium hydroxide (using as little as possible) followed by hot water, allowing the solution to run into the flask, thus dissolving the adhering precipitate. Wash the paper very thoroughly with hot water. Transfer the entire solution and washings to a 250-cc beaker of resistance glass. The total volume of the solution should not be greater than 100 cc. Nearly neutralize with hydrochloric acid, the reformation of the yellow precipitate serving as indicator. Redissolve the precipitate that finally forms by the addition of a few drops of dilute ammonium hydroxide. Cool and add, very slowly and with vigorous stirring, 25 cc of magnesia mixture. After 15 minutes add ammonium hydroxide (specific gravity 0.90) equal to one-ninth of the total volume of the solution, stirring as this is added. Cover and allow to stand for two hours. Filter and wash with dilute ammonium hydroxide (*a*) until practically free from chlorides, as shown by acidifying the washings with nitric acid and adding silver nitrate solution. Dry the filter and precipitate and transfer the latter to a porcelain crucible, previously ignited and weighed. Ignite the filter separately and transfer its ash, when white, to the crucible containing the main precipitate. Ignite to whiteness or grayish white over the blast lamp or Méker burner, weigh and calculate the per cent of phosphorus pentoxide.

Volumetric Determination.—Have the following solutions ready:

(*a*) *Ammonium Molybdate.*—To each 100 cc of the molybdate solution that was prepared for the gravimetric determination of phosphorus add 5 cc of concentrated nitric acid. The solution should be filtered immediately before using.

(*b*) *Standard potassium hydroxide solution,* 1 cc of which is equivalent to 1 mg of phosphorus. (Refer to equation (2) on page 91, Part I.) This should be as nearly free from carbonates as possible and is made as follows: Dissolve 2 per cent more than the calculated quantity for 1000 cc, dilute to 100 cc and add 1 cc of a saturated solution of barium hydroxide. Stopper the flask and allow to stand until the precipitate of barium carbonate has settled. Decant and dilute to 1000 cc. Standardize by titration against solution (*c*), using phenolphthalein. Adjust so that 1 cc is equivalent to 0.1 mg of phosphorus.

(*c*) *Standard Hydrochloric or Nitric Acid.*—This solution should be equivalent in strength to the standard base. It should be made from previously boiled and cooled water and it should be standardized by titration against the basic solution, using phenolphthalein as indicator.

The fertilizer is dissolved by either of methods (*b*), (*e*), (*f*) or (*g*), page 276. Method (*e*) is to be preferred if the material will yield to this treatment. The solution is to be diluted and filtered as already directed.

In the case of fertilizers containing less than 5 per cent of phosphorus pentoxide, use an aliquot corresponding to 0.4 gm of substance. If the percentage is between 5 and 20 use an aliquot corresponding to 0.1 gm of substance.

Add 5 to 10 cc of concentrated nitric acid, the amount depending upon whether this acid has been used in making the solution; or add ammonium nitrate equivalent to this amount of nitric acid. Nearly neutralize with ammonium hydroxide, precipitation of hydroxide of iron or aluminum serving as indicator. Clear with a drop of nitric acid, dilute to about 100 cc and heat by immersing in water at 60° to 65°. For phosphorus pentoxide per cents below 5 add 25 cc of freshly filtered molybdate solution; for percentages between 5 and 20 add 35 cc of molybdate solution. For percentages greater than 20 add sufficient molybdate solution to insure complete precipitation of the phosphorus. Stir, allow to stand in the bath for 15 minutes and filter at once. Wash twice with water by decantation, using 25 to 30 cc each time and agitating and settling each time before decanting. Transfer the precipitate to the filter as thoroughly as can be done without the use of a policeman and wash the flask, paper and precipitate with cold, recently boiled water until the filtrate from two fillings of the filter yields a pink color upon the addition of phenolphthalein and one drop of the standard base. Remember that a trace of acid left in any of these materials will vitiate the results of the titration.

Return the filter paper and precipitate to the flask in which precipitation was made. Add a measured, small excess of the standard base to dissolve the yellow precipitate, then add a few drops of phenolphthalein and titrate the unused excess of base with standard acid. Calculate the per cent of phosphorus pentoxide in the sample.

The following changes in the method just described are made *optional:*

(*a*) Heat the solution to only 45° to 50° and allow to stand in the bath, after the addition of the molybdate solution, for 30 minutes.

(*b*) Cool to room temperature before adding the molybdate solution. Add the latter at the rate of 75 cc for each decigram of phosphorus pentoxide present, place the stoppered flask containing the solution in a mixing apparatus (Fig. 51) and mix for 30 minutes at room temperature. Filter at once and proceed as already directed.

Determination of Water-soluble Phosphorus: *Gravimetric Method.*—Place an accurately weighed 2-gm sample on a filter and wash with small portions of cold water until about 250 cc of washings has been obtained. Allow each portion of water to run through before adding another. Keep the residue for the determination of citrate-insoluble phosphorus. Dilute the filtrate to exactly 500 cc and mix.

Place 50-cc aliquots in flasks, add 10 cc of concentrated nitric acid and then ammonium hydroxide until a slight permanent precipitate is formed. Clear with a few drops of nitric acid, dilute to about 100 cc and determine the water-soluble phosphorus gravimetrically as in the case of total phosphorus. Report as phosphorus pentoxide of water-soluble compounds.

Volumetric Determination.—Wash 2 gm of the sample as directed above for the gravimetric method. Measure the aliquot of the filtrate, and neutralize as there directed. Dilute to 60 cc and precipitate the phosphorus as directed for the volumetric determination of total phosphorus. Calculate the per cent of phosphorus pentoxide of water-soluble compounds.

Citrate-insoluble Phosphorus.—The value of a fertilizer is frequently rated upon the degree of solubility or the availability of its constituents to plants, as already explained. For this purpose it is desirable to imitate the solvent action of solutions found in soils. The use of ammonium citrate solution provides an approximate distinction between available and non-available phosphates, although it should be noted that there is still considerable disagreement among agricultural chemists as to the true availability of the different compounds of phosphorus. The solvent action of this solution upon calcium phosphate is largely due to the presence of free citric acid or of acid citrates, caused by the hydrolysis of the ammonium citrate (*i.e.*, to the fact that chemically equivalent quantities of ammonium hydroxide and citric acid in solution yield an acid condition, P_H being less than 7).

$$(NH_4)_3C_6H_5O_7 + H_2O \rightarrow (NH_4)_2HC_6H_5O_7 + NH_4OH \quad (1)$$

$$(NH_4)_3C_6H_5O_7 + 2H_2O \rightarrow NH_4H_2C_6H_5O_7 + 2NH_4OH, \quad (2)$$

$$(NH_4)_3C_6H_5O_7 + 3H_2O \rightarrow H_3C_6H_5O_7 + 3NH_4OH, \quad (3)$$

$$2CaHPO_4 + 2H_3C_6H_5O_7 \rightarrow Ca(H_2PO_4)_2 + Ca(H_2C_6H_5O_7)_2. \quad (4)$$

Hydrolysis of ammonium citrate is due to the fact that both ammonium hydroxide and citric acid are weak electrolytes. Because of this fact it is very difficult to prepare a solution in which the two electrolytes are present in exactly equivalent quantities (a "neutral" solution), using an indicator to determine this condition. It is well to remember that the citrate is always largely hydrolyzed and that it is, therefore, rather a solution of (at best) equivalent quantities of the two constituents, acid and base.

Ammonium Citrate Solution.—Two methods are approved by the A. O. A. C. for preparing "neutral" ammonium citrate. In one of them a stated amount of citric acid in solution is neutralized by ammonium hydroxide, using corallin (rosolic acid) as indicator. This method is unreliable because corallin is not sufficiently sensitive to citric acid or ammonium hydroxide.

In the other method the solution is nearly neutralized and a small excess of calcium chloride solution in water and alcohol is added. Calcium citrate, a salt of small solubility, precipitates as a result of such reactions as the following:

$$2(NH_4)_3C_6H_5O_7 + 3CaCl_2 \rightarrow Ca_3(C_6H_5O_7)_2 + 6NH_4Cl, \qquad (1)$$

$$2(NH_4)_2HC_6H_5O_7 + 3CaCl_2 \rightarrow Ca_3(C_6H_5O_7)_2 + 4NH_4Cl + 2HCl, \qquad (2)$$

$$2NH_4H_2C_6H_5O_7 + 3CaCl_2 \rightarrow Ca_3(C_6H_5O_7)_2 + 2NH_4Cl + 4HCl, \qquad (3)$$

$$2H_3C_6H_5O_7 + 3CaCl_2 \rightarrow Ca_3(C_6H_5O_7)_2 + 6HCl. \qquad (4)$$

Equation (1) shows that if only triammonium ("neutral") citrate is present, no matter how highly this may be hydrolyzed, the solution will be left neutral to all indicators by the removal of calcium citrate. According to Eqs. (2), (3) and (4) any acid citrate or free citric acid will produce free hydrochloric acid, which may be made evident by the use of indicators. On the other hand, if the citrate solution contained an excess of ammonium hydroxide this would remain after the precipitation of calcium citrate. According to the result obtained by testing the filtrate with an indicator, either citric acid or ammonium hydroxide may be added, as necessary, to obtain the proper condition of equivalent quantities of acid and base. That this solution is not really neutral and that it does not really contain triammonium citrate, has already been explained.

Preparation of Ammonium Citrate Solution: *Calcium Chloride Method.*—To 370 gm of commercial citric acid, dissolved in 1500 cc of water, add commercial ammonium hydroxide until nearly neutral, testing with recently prepared corallin solution. Add water until the specific gravity is about 1.11 at 20°

Prepare a solution of fused calcium chloride, 20 gm to 100 cc, and add 400 cc of 95-per cent alcohol. Make this solution exactly neutral with tenth-normal ammonium hydroxide or hydrochloric acid, as may be necessary, using freshly prepared corallin solution as a preliminary indicator; test finally by diluting 2 cc with an equal volume of water and adding methyl red (cochineal is the official indicator for this purpose). Approximately 50 cc of this solution will precipitate the citric acid from 10 cc of the citrate solution.

To 10 cc of the nearly neutral ammonium citrate solution, add 50 cc of the alcoholic calcium chloride solution, stir well and filter at once through a folded filter. Dilute the filtrate with an equal volume of water and test the reaction with a neutral solution of methyl red (or cochineal). If basic or acid, add citric acid or ammonium hydroxide, as the case may be, to the main portion of the citrate solution. Mix and test again as before. Repeat this process until a neutral reaction of the filtrate is obtained. Add sufficient water to make the specific gravity 1.09 at 20°.

Determination of Citrate-insoluble Phosphorus: *Acidulated Samples.*—Heat 100 cc of ammonium citrate solution to 65° in a 250-cc Erlenmeyer flask placed in a water bath at this temperature, keeping the flask loosely stoppered to prevent evaporation. Use a thermometer in both flask and bath. The level of the water in the bath should be above that of the citrate solution in the flask. When the temperature of the citrate solution has reached 65°, drop into it the filter containing the washed residue obtained in the determination of water-soluble phosphorus (page 278), or a weighed 2-gm sample of the original fertilizer, if water-soluble phosphorus is not to be determined. Close the flask tightly with a smooth rubber stopper and shake violently until the filter paper is reduced to a pulp (or for 2 or 3 minutes if no paper has been used), occasionally relieving the pressure by momentarily removing the stopper. Place the flask in the bath and maintain its contents at exactly 65°. Shake the flask every 5 minutes. At the expiration of 30 minutes from the time the filter and residue were introduced, remove the flask from the bath and immediately filter the contents as rapidly as possible through quick-acting filter paper. Wash with recently boiled water at 65° until the volume of the filtrate is about 350 cc, allowing time for thorough draining each time before adding new portions of water. Either (1) transfer the filter and its contents to a crucible, ignite until the organic matter is destroyed, add 10 to 15 cc of concentrated hydrochloric acid and digest until all the phosphate is dissolved; or (2) transfer the filter with contents to a digestion flask, add 35 cc of concentrated nitric acid and 10 cc of concentrated hydrochloric acid and warm until the phosphate is dissolved. There may be an insoluble residue of silicates or silica in either case. Ten minutes of digestion in the warm acid should be sufficient to dissolve all phosphates.

Dilute the solution as prepared in (1) or (2) to 200 cc. Mix well, filter through a dry filter and determine the phosphorus as already directed for total phosphorus, pages 276 to 278. Calculate the per cent of citrate-insoluble phosphorus, as pentoxide, deduct from the per cent of total phosphorus pentoxide and report the remainder as the per cent of available phosphorus pentoxide.

Non-acidulated Samples.—Treat 2 gm of the material, without previous washing with water, as directed for acidulated samples, unless the substance contains much animal matter (such materials as fish and bone) in which case dissolve the residue insoluble in ammonium citrate by one of the processes (*b*), (*c*) or (*d*), page 276. Determine as directed for total phosphorus.

Nitrogen.—Nitrogen is one of the most important of the elements that are concerned in plant growth. Although abundant in the atmosphere in an uncombined form, it is an expensive element when used in making up a fertilizer. This is because its inert nature makes difficult the problem of forming nitrogen compounds which may be used by plants. Nitrogen should therefore be obtained, so far as possible, through growing inoculated legumes in rotation, rather than through purchase in the form of fertilizers.

Nitrogen is usually present in a fertilizer in one or more of the following forms: (1) Ammonium salts, such as ammonium sulphate or nitrate; (2) animal or vegetable matter, such as dried blood, cotton seed meal, stable manure and guano; (3) atmospheric nitrogen fixed by electrical energy, as various nitrates. Sodium nitrate is found also as a natural product, chiefly in South America.

Organic fertilizers have some advantages over the others in that they promote bacterial action. Because of their limited solubility they do not readily leach out of the soil, the result being that they are used less rapidly and supply the plant with nitrogen through a longer period of growing season. Calcium cyanamid also acts like the organic forms as it slowly breaks down in the soil, somewhat as follows:

$$\underset{\text{Calcium cyanamid}}{CaNCN} + CO_2 + 2H_2O \rightarrow CaCO_3 + \underset{\text{Urea}}{CO(NH_2)_2}, \qquad (1)$$

$$CO(NH_2)_2 + 2H_2O \rightarrow (NH_4)_2CO_3\,, \qquad (2)$$

ammonium carbonate being available to plants.

Nitrogen used in the form of ammonium sulphate has not the most desirable action, as it finally leaves free acid in the soil, due to hydrolysis and absorption of the resulting ammonia. Chili saltpeter (sodium nitrate) has the opposite effect in the soil as the nitric acid formed by hydrolysis is used, leaving sodium hydroxide which lessens the acidity of the soil or even causes a basic condition. This is sometimes desirable, although excessive basicity may change the texture of the soil because of the deflocculating effect upon the clay particles, thus resisting the penetration of rain water and the normal movements of drainage water. This was illustrated in the experiment on deflocculation, page 268.

Because of the differences in cost and availability of different forms of nitrogen, it is often desirable to know the relative amounts existing as nitrates, ammonia or organic forms in the fertilizer. The following methods will give information of this character.

Detection of Nitrates.—If sulphuric acid is added to a nitrate, nitric acid will be set free. This will be reduced to nitric oxide in the presence of ferrous sulphate, forming a brown ring ($FeSO_4 \cdot N_2O_2$ or $FeSO_4 \cdot NO$).

Treat 5 gm of fertilizer with 25 cc of hot water, then filter. Mix about 3 cc of this solution with an equal volume of concentrated sulphuric acid (free from nitrates) in a test tube and cool, then pour 2 or 3 cc of concentrated ferrous sulphate solution carefully down the side of the tube so that the two liquids do not mix. In the presence of nitrates a brown or reddish brown ring will form at the junction between the two solutions. If no color forms immediately let stand 2 or 3 minutes.

Nitrogen of Ammonium Salts.—If a material containing nitrogen in various forms is placed in water and heated with magnesium oxide, ammonia is distilled and both nitrates and protein nitrogen remain behind. Magnesium hydroxide is the active agent:

$$MgO + H_2O \rightarrow Mg(OH)_2, \qquad (1)$$

$$Mg(OH)_2 + 2NH_4NO_3 \rightarrow Mg(NO_3)_2 + 2NH_3 + H_2O. \qquad (2)$$

The ammonia is absorbed in standard acid and the titration finished as usual.

Determination of Ammonia Nitrogen: *Magnesium Oxide Method.*—Place 2 gm of sample in a Kjeldahl digestion flask with about 200 cc of water and 5 gm or more of magnesium oxide which has been rendered free from carbonates by a previous strong ignition. Connect the flask with a condenser and distill 100 cc of the liquid into 50 cc of fifth-normal acid. Titrate the excess with fifth-normal base solution, using methyl red. Calculate the per cent of ammonia nitrogen.

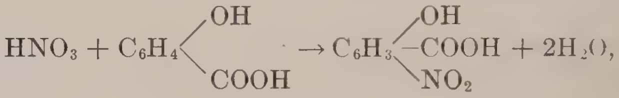
Determination of Organic and Ammonia Nitrogen: *Kjeldahl Method.*—The method described for organic nitrogen in feeds, page 151, includes also nitrogen of ammonium salts if present, as they may be in fertilizers. Determine as there directed, using accurately weighed samples of about 2 gm.

Determination of Organic and Ammonia Nitrogen: *Gunning Method.*—Determine as for organic nitrogen in feeds, page 154.

Nitrate Nitrogen.—When nitrogen is determined by these methods most of the nitrate nitrogen is volatilized and lost upon digesting with sulphuric acid. In order to avoid this loss the Kjeldahl method may be modified by adding benzoic acid, then using permanganates to oxidize the nitrobenzoic acid to ammonia. Phenolsulphonic acid may be substituted for benzoic acid, the nitrophenolsulphonic acids formed being then reduced to aminophenolsulphonic acid by zinc dust. This compound is then oxidized by heating with sulphuric acid.

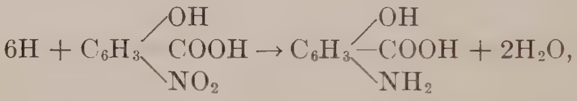
Salicylic acid has now superseded both benzoic acid and phenolsulphonic acid. The reducing agent is either sodium thiosulphate or zinc dust:

$$2KNO_3 + H_2SO_4 \rightarrow K_2SO_4 + 2HNO_3, \qquad (1)$$



$$HNO_3 + C_6H_4\left\langle\begin{matrix}OH\\ \\ COOH\end{matrix}\right. \rightarrow C_6H_3\left\langle\begin{matrix}OH\\ -COOH\\ NO_2\end{matrix}\right. + 2H_2O, \qquad (2)$$

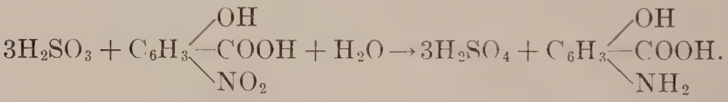
The nitro compound is then reduced by nascent hydrogen from zinc and sulphuric acid:



$$6H + C_6H_3\left\langle\begin{matrix}OH\\ COOH\\ NO_2\end{matrix}\right. \rightarrow C_6H_3\left\langle\begin{matrix}OH\\ -COOH\\ NH_2\end{matrix}\right. + 2H_2O, \qquad (3)$$

or by sodium thiosulphate:

$$Na_2S_2O_3 + H_2SO_4 \rightarrow Na_2SO_4 + H_2SO_3 + S, \qquad (4)$$



$$3H_2SO_3 + C_6H_3\left\langle\begin{matrix}OH\\ -COOH\\ NO_2\end{matrix}\right. + H_2O \rightarrow 3H_2SO_4 + C_6H_3\left\langle\begin{matrix}OH\\ -COOH.\\ NH_2\end{matrix}\right. \qquad (5)$$

The amino acid is then oxidized by concentrated sulphuric acid, ammonium sulphate resulting.

Determination of Total Nitrogen in Materials Containing Nitrates: *Modified Kjeldahl Method.*—Weigh 2 gm of fertilizer and place in a Kjeldahl flask. Add 30 cc of concentrated sulphuric acid containing 2 gm of salicylic acid (these must be added together) and mix by shaking vigorously. After 30 minutes add 5 gm of sodium thiosulphate or 2 gm of zinc dust. If zinc dust is used it must be added gradually, shaking the flask after each addition. Heat gently until frothing ceases then boil for 10 minutes. Add 0.7 gm of mercury oxide or 0.3 gm of copper sulphate and continue the digestion, distillation and titration as in the Kjeldahl method. Make a blank determination for nitrogen in the reagents, using sugar as already directed. Calculate the per cent of total nitrogen in the fertilizer.

Nitrate and Ammonia Nitrogen.—These two forms of nitrogen may be determined together by first reducing the nitrate to ammonia by nascent hydrogen, then distilling the solution made basic by magnesium hydroxide.

Determination of Nitrate and Ammonia Nitrogen: *Iron Reduction Method.*—Place 1 gm of the sample in a 500-cc flask, add about 30 cc of water and 3 gm of iron reduced by hydrogen. After standing long enough to insure solution of nitrates and ammonium salts, add 10 cc of a mixture of equal volumes of concentrated sulphuric acid and water; shake thoroughly, place a funnel in the neck of the flask to prevent mechanical loss and allow to stand until the reaction has moderated. Heat the solution slowly, then boil for 5 minutes and cool. Add about 100 cc of water, a little paraffin to prevent foaming and 10 gm of magnesium oxide, made free from carbonates by previous strong ignition. Connect with the tin condenser and boil for 40 minutes, or nearly to dryness, collecting the distillate in 50 cc of fifth-normal acid. Titrate the excess of acid with fifth-normal base, using methyl red, and calculate nitrogen of nitrates and ammonia.

If the sample is known to consist of nitrates alone, proceed as above except that 0.25 gm of the sample, is used, together with 5 gm of reduced iron. After the boiling, add 75 cc of water and an excess of saturated sodium hydroxide solution (instead of magnesium oxide), and distill as above directed.

Availability of Nitrogen.—Mention has already been made of the low fertilizing value of certain nitrogenous materials, due to slowness of decomposition occurring when the fertilizer is added to the soil. Nitrogen is probably directly assimilated by plants only in the most highly oxidized form, *i.e.*, that of nitrates. Ammonium salts and certain organic materials, such as dried blood, have almost as great value because they readily decompose and oxidize in the soil, forming nitrates. Hoof, hair, leather and hide are rich in nitrogen but they do not so decompose, except very slowly, and a method for differentiating between available and non-available forms of nitrogen is desirable. The microscope will detect ground hair and other similar materials but it can give only qualitative results. Fortunately qualitative results are all that are necessary where the addition of such materials is contrary to law, but for scientific purposes a quantitative distinction between available and non-available nitrogen may be of great practical use. An exact analytical method for such a purpose seems to be impossible because there is no sharp distinction to be made between the classes of fertilizer materials.

Great reliance is placed upon culture experiments, comparing the effect of using different fertilizers with plants under otherwise identical conditions. However, such experiments are slow and they have no value whatever for analytical purposes. An approximate distinction can be made by the use of potassium permanganate in either neutral or basic solution. Readily decomposable materials are oxidized and the nitrogen is converted into ammonia. It is not yet entirely clear as to how much reliance is to be placed upon these methods but they have been adopted by the Association of Official Agricultural Chemists.

Determination of Total Water-insoluble Organic Nitrogen.—Place 1 gm of the material upon an 11-cm filter paper and wash with recently boiled water at room temperature until the filtrate measures 250 cc. Dry and determine nitrogen in the residue by the Kjeldahl method, making a blank determination to correct for the nitrogen of the filter paper.

Determination of Water-insoluble Organic Nitrogen, Soluble in Potassium Permanganate.—Place a weighed quantity of the fertilizer, equivalent to 50 mg of the water-insoluble organic nitrogen as determined above, on a moistened 11-cm filter paper and wash with recently boiled water at room temperature until the filtrate measures 250 cc. Transfer the insoluble residue with 25 cc of water (at about 30°) to a 400-cc low-form beaker, add 1 gm of sodium carbonate, mix and add 100 cc of 2-per cent potassium permanganate solution. Cover with a glass and immerse for 30 minutes in a water or steam bath so that the level of the liquid in the beaker is below that of the heating medium. Keep at 100°, stirring twice at intervals of 10 minutes each. At the end of this time remove from the bath, add immediately 100 cc of cold water and filter through a heavy 15-cm folded filter. Wash with small quantities of cold water until the filtrate measures about 400 cc. Determine total nitrogen in the residue and filter by either of the methods already described (not modified for nitrates) making a blank determination to correct for the nitrogen contained in the filter. The nitrogen thus obtained is the *inactive water-insoluble organic nitrogen*. Subtract this per cent from the total water-insoluble organic nitrogen. The remainder is the per cent of *organic nitrogen soluble in neutral permanganate*. As already explained, this is an approximate measure of organic nitrogen easily available for plant food.

Determination of Organic Nitrogen Soluble in Basic Permanganate.—Prepare a solution of potassium permanganate by dissolving 25 gm in about 100 cc of water; dissolve 150 gm of sodium hydroxide in 500 cc of water and, after this has cooled, mix with the potassium permanganate solution and dilute the whole to 1000 cc.

(*a*) *Mixed Fertilizers.*—Place an amount of material equivalent to 50 mg of total water-insoluble organic nitrogen, determined as above, on a filter paper and wash with water at room temperature until the filtrate measures about 250 cc.

(*b*) *Raw Materials.*—Place an amount of material equivalent to 50 mg of total water-insoluble organic nitrogen, determined as above, in a small mortar. Add about 2 gm of powdered rock phosphate (to facilitate the washing process) mix thoroughly by grinding, transfer to a filter paper and wash with water at room temperature until the filtrate measures 250 cc. When much oil or fat is present, it is well first to wash several times with ether and to allow to stand until the odor of the latter has disappeared before extracting with water.

Dry the residue from either class of materials at a temperature not exceeding 80° and transfer from the filter to a 500-cc Kjeldahl digestion flask. Add 20 cc of water, about 1 gm of crushed porcelain to prevent bumping and about 1 gm of paraffin to prevent frothing. Add 100 cc of the basic permanganate solution and connect with the tin condenser, the lower end of which dips into 50 cc of fifth-normal acid.

Digest slowly for at least 30 minutes, below the distillation point, with a very low flame, using wire gauze and asbestos paper between the flask and flame. Gradually raise the temperature and, after any danger of frothing has passed, distill until 95 cc of the distillate (145 cc of distillate plus acid) is obtained, then titrate as usual. If a tendency to froth is noticed lengthen the digestion period. During the digestion gently rotate the flask occasionally, particularly if the material shows a tendency to adhere to the sides of the flask.

The nitrogen thus obtained is the *active water-insoluble organic nitrogen.*

Potassium.—Most soils contain orthoclase (potassium aluminium silicate) but the potassium of this is unavailable or so slowly available that the supply from this source is often not sufficient to meet the needs of the rapidly growing plant. The need is especially great in muck soils for plants such as potatoes or sugar beets, which require a large amount of potassium.

Sodium compounds can take the place of potassium to only a very slight extent, if at all. It has been noted that in places where sodium nitrate has been used for some time to supply nitrogen, much less than the usual response could be obtained from potassium fertilizers. It is assumed therfore, that the sodium of the fertilizer tended partly to perform the function of potassium. The effect of potassium starvation is more definite than that resulting from phosphorus deficiency and it is indicated by the color of the plant becoming abnormal and dull, the stem weak and the ability to manufacture starch at the normal rate lacking.

Preparation of Fertilizer Solution: (*a*) *Mixed Fertilizers.*—Place 25 gm of the sample upon a 12.5-cm filter paper and wash with boiling water until the filtrate measures about 200 cc. Add to the filtrate 2 cc of concentrated hydrochloric acid, heat to boiling, transfer to a 250-cc volumetric flask and add to the hot solution a slight excess of ammonium hydroxide and sufficient ammonium oxalate to precipitate all of the calcium. Cool, dilute to 250 cc, mix and pass through a dry filter. Reject the first 25 cc of the filtrate.

(*b*) *Simple Potassium Salts, Potassium Magnesium Sulphate and Kainite.*—Dissolve 2.5 gm of sample in a 250-cc volumetric flask and dilute to the mark without the addition of ammonium hydroxide or ammonium oxalate.

(*c*) *Organic Compounds: Cotton Seed Meal, Tobacco Stems, Etc.*—Saturate 10 gm of sample with concentrated sulphuric acid, then evaporate and ignite at a temperature not above that of dull redness to destroy organic matter. A muffle furnace will be found to be convenient for this operation. Add a little concentrated hydrochloric acid and warm slightly in order to loosen the mass from the dish. Wash into a 500-cc volumetric flask, add ammonium hydroxide and ammonium oxalate to precipitate calcium, dilute to the mark and mix well. Filter through a dry paper and reject the first 25 cc of the filtrate.

(*d*) *Ashes from Wood or Cotton Hulls.*—Digest 10 gm with 300 cc of boiling water for 30 minutes in a covered flask. Precipitate calcium with ammonium hydroxide and ammonium oxalate, as directed under (*a*), above, rinse into a 500-cc flask, dilute to the mark and mix well. Filter through a dry paper and reject the first 25 cc of the filtrate.

Determination of Potassium: (*a*) *In Mixed Fertilizers and Ashes.*—The principles underlying the determination of potassium have been discussed under the head of soil analysis, page 244. Directions for the preparation of chlorplatinic acid solution and of 80-per cent alcohol also have been given. Prepare, in addition, a 20-per cent ammonium chloride solution, saturated with potassium chlorplatinate by agitating occasionally for several hours, after having added about 10 gm of the salt for each 500 cc of solution. Allow to settle and then filter.

Evaporate 50 cc of the prepared solution nearly to dryness in a dish, add 1 cc of sulphuric acid (1 to 1), evaporate to dryness and ignite at a dull red heat until organic matter is removed and the residue is white. Dissolve the residue in hot water, using at least 20 cc for each decigram of potassium oxide present, add a few drops of concentrated hydrochloric acid and enough chlorplatinic acid to precipitate all of the potassium and to leave about 1 cc of platinum solution in excess. If the per cent of potassium is approximately known the quantity of platinum solution that is necessary should be calculated. Contamination with ammonia vapor must be avoided.

Evaporate the solution on a steam bath to a thick paste, cool and add to the residue 25 cc of 80-per cent alcohol. Stir thoroughly and allow to stand for 15 minutes. Filter through a weighed Gooch crucible. If the filtrate is not colored, sufficient chlorplatinic acid solution is not present and the analysis must be begun again with another portion of the solution, increasing the amount of platinum solution.

Wash the precipitate with 80-per cent alcohol, continuing the washing after the filtrate has become colorless. Remove the filtrate and washings to the bottle which has been provided for waste platinum solutions and wash the precipitate five times with 10-cc portions of the ammonium chloride solution. Wash again thoroughly with 80-per cent alcohol, exercising particular care to remove ammonium chloride from the upper part of the filter. Dry the precipitate for 30 minutes at 100°, cool and weigh. The weight of potassium chlorplatinate is given without further treatment. The precipitate should be completely soluble in warm water.

(*b*) *In Commercial Potassium Chloride* (*"Muriate of Potash"*).—To 50 cc of the solution already prepared add a few drops of hydrochloric acid and 10 cc of chlorplatinic acid solution. Evaporate over a steam bath to a thick paste and treat the residue as in the case of mixed fertilizers.

(*c*) *In Potassium Sulphate, Potassium Magnesium Sulphate and Kainite.*—Acidify 50 cc of the solution with a few drops of hydrochloric acid, add 15 cc of chlorplatinic acid solution and evaporate on the steam bath to a thick paste. From this point proceed as with mixed fertilizers, except that 25 cc portions of the ammonium chloride solution should be used in the washing process.

The potassium is reported as per cent of potassium oxide (often called "potash") instead of as the element.

Perchlorate Method.—In the discussion of methods for the determination of potassium in soils, page 244, attention was called to the fact that the increasing price of platinum has greatly handicapped laboratory work of this character and that methods not requiring the use of platinum solutions are rapidly increasing in importance. The perchlorate method as described for soil work is adapted also to fertilizer investigations. The solutions of potassium, obtained by extraction of the fertilizer for determinations by the chlorplatinate method, may be used for this purpose, the determination of potassium in these being performed exactly as directed for potassium in soils.

Centrifugal Method.—There is need for a short approximate method for determining potassium which will fill somewhat the same place as the Babcock method for determining fat in cream and milk. A method has been devised by Sherrill[1] which is based upon a comparison of the volumes of precipitates of potassium cobaltic nitrite formed from two solutions—the potassium concentration of one being known. The precipitates are separated into graduated tubes by centrifugal action and the volumes noted. The method seems to be fairly accurate and it is useful when a rapid determination for factory control is necessary.

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[1] *J. Ind. Eng. Chem.*, **13**, 227 (1921).

One of the writers has had some experience with this method for determining potassium in fertilizers of various kinds, and it has been found possible to check reasonably well with the results obtained by the chlorplatinate method. Some results obtained by the two methods are given in the following table:

TABLE XXV.—COMPARISON OF THE PER CENT OF POTASSIUM OXIDE IN FERTILIZERS BY CENTRIFUGAL AND CHLORPLATINATE METHOLS

Sample No.	Chlorplatinate method*	Centrifugal method†
1581	3.12	3.2
1604	1.74	1.8
1669	3.67	3.8
1823	8.04	8.2
1949	4.26	4.4
2176	9.38	9.1
2224	50.25	50.1
1979	4.83	4.9

* By the Indiana State Chemist.

† By one of the authors.

Special bottles have been described by Sherrill for this determination. These are of the form shown in Fig. 60. Or the older Goetz bulbs, as used for rapid determinations of phosphorus in steel, will be found convenient. The precipitate is collected and measured in the narrow, graduated portion of the tube.

If the potassium solution contains ammonia or ammonium salts, these must be expelled by evaporating a measured portion to a small volume with enough sodium hydroxide to render the solution decidedly basic, or by evaporating to dryness and igniting at dull redness. The solution is then acidified with acetic acid and diluted to the original volume.

Determination of Potassium: *Centrifugal Method.*—Prepare solutions as follows:

(*a*) *Standard Potassium Chloride Solution.*—Dissolve 15.83 gm of pure potassium chloride in distilled water, add ten drops of glacial acetic acid and dilute to 1000 cc. This makes a solution containing 1 per cent of potassium oxide.

(*b*) *Sodium Cobaltic Nitrite Solution.*—Dissolve 225 gm of sodium nitrite in 400 cc of distilled water. Also dissolve 125 gm of cobalt acetate crystals in 400 cc of water. Mix the solutions, dilute to 1000 cc and mix. To 100 cc of this solution add 65 cc of distilled water and 5 cc of glacial acetic acid, mix and allow to stand over night. This diluted solution is unstable and it should not be kept for use more than five days.

Measure 17 cc of solution (*b*) into each tube, the temperature being not lower than 22°. Be sure that the graduated stems are filled, with entire absence of air bubbles. To one of the tubes add 5 cc of solution (*a*) and to each of the others 5 cc of the diluted sample. Whirl immediately for one minute at the rated speed for the centrifuge that is being used. Remove the tubes and tap those in which the upper surface of the column of precipitate is not practically plane. Whirl again for 15 seconds. The reading of the precipitate in the tubes containing the sample solutions should be within 5 divisions (either way) of that of the standard. If this is not the case, repeat the experiment, using more concentrated or more dilute solutions, as indicated.

From the relative volumes of the precipitates and the known potassium content of the standard solution, calculate the per cent of potassium (or of potassium oxide) in the sample.

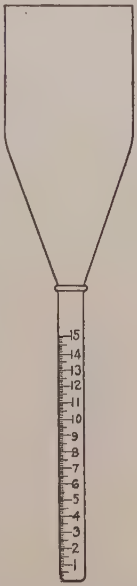

FIG. 60.—Graduated tube for determination of potassium by the Sherrill centrifugal method.

Methods of Pot and Field Culture.—From analyses alone it is difficult to foretell just what will be the response of a plant to any given application of fertilizer to a soil. The great variety of soil components, including toxic substances often contained in them, is responsible for this, and it is very desirable that pot and field tests be conducted for the purpose of gaining more information as to the needs of the soil for the growth of any particular crop. This is analogous to conducting feeding experiments with animals for testing the degree of utilization and the physiological effects of the feeds.

Much valuable information has been gained through sand and water culture experiments, in which solutions of certain compounds are added. Reference may be made to the experiments of Knop,[1] Shives[2] and Tottingham.[3]

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[1] *Landw. Vers. Sta.*, **7,** 93 (1865).

[2] *N. J. Exp. Sta. Bull.*, **319** (1917).

[3] *J. Am. Soc. Agron.*, **2,** 1 (1919).

CHAPTER XIV

INSECTICIDES AND FUNGICIDES

The large number of insect and fungus pests with which the economic entomologist and the horticulturist have had to contend in recent years has caused a renewed search for methods for more efficient control. The insecticides used for this purpose belong to one of two classes, depending upon whether they are for external or internal action. Paris green and London purple are examples of those of internal application, while lime-sulphur mixture and kerosene emulsion are examples of those designed to kill by contact. Bordeaux mixture is a well known remedy for fungus pests.

Character of Insecticide as Related to Insect Anatomy.—There is a close relation between the general character of the insecticide sprays to be applied and that of the mouth parts of insects. Generally speaking, insects secure their food either by biting out and swallowing plant particles or by sucking juices from interior portions of the plant. Those of the biting kind have jaws and also certain accessory parts which enable the insect to cut and pass on the small parts of food to the digestive organs. Most sucking insects have mouth parts of long bristle-like structure. These are inclosed in a tube and the bristles and beak together constitute a sucking apparatus for the extraction of the plant juices. It is possible to kill both sucking and biting insects by poisoning the air with hydrocyanic acid or other poisonous gases, as well as by poisons that are to be eaten by the insect.

Action of Contact Insecticides.—Considerable attention has been given to the method by which contact insecticides kill. Shafer[1] found that in the case of certain volatile insecticides, such as gasoline, carbon disulphide or chloroform, the fatty membranes absorb some of the vapor, which renders them less permeable to oxygen. The cells thus gradually cease to function in a normal way. Non-volatile insecticides in the form of powdered solids may function by sticking to certain body secretions, then being absorbed into the tissues. As examples of this class, borax and sodium fluoride are frequently used to exterminate cockroaches. The powder sticks to the body of the insect and is partly absorbed but it also acts as a stomach poison because some of it is usually licked off and swallowed by the animal.

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[1] *Mich. Exp. Sta. Tech. Bull.*, **21** (1915).

The vapor of white hellebore is insufficient to kill insects but Shafer has noted that rose slugs which come into contact with this insecticide gradually become numbed and fall from the leaves. This occurred even in cases where none of the insecticide had been eaten. It is concluded that the numbing effect is due to slight dissolving of the powder and surface absorption by the excretions, little if any of the insecticide passing through the cuticular covering, and that the cause of the final death of the insect is due more to drying and starving than to any other reason.

Finally, the natural cells of some insects contain enzymes, the normal functioning of which is of the greatest importance to the well-being of the insect. The interference of the various insecticides with the activity of these enzymatic bodies is known to be serious and this may be the cause of the death of the insect in some cases.

Preparation of Insecticides.—The internal poisons are usually prepared in considerable quantities and their preparation should be under chemical control. The contact and fungicide poisons are freshly prepared by the sprayer and their efficiency depends upon the composition and proportions of the ingredients. Arsenic has been so universally used as an active internal poison for insects that the determination of this element is highly important. Free arsenous acid in solution has a destructive action on foliage, therefore it is necessary also to limit the per cent of arsenic in this form. The maximum quantity which is safe for foliage varies from 4 to 6 per cent.

Mixing of Sprays.—The question of combining insecticides and fungicides for the control of orchard pests is important from the standpoint of saving time and money as well as from that of increasing the efficiency of the spray. Choosing the proper spray materials and mixing them so as to retain their insecticidal or fungicidal value is often a difficult and complicated problem.[1] Chemical or physical changes may take place on mixing, resulting in compounds being formed which are injurious to foliage, or in some cases the spray may become worthless because the active killing agent has become inert.

The various objectionable combinations are shown in Table XXVI on page 295. It will be noted that Bordeaux mixture (copper sulphate and calcium hydroxide) with arsenate of lead is permissible. It has been shown by analysis that when these are mixed the amount of soluble arsenic is not much greater than when lead arsenate is treated with pure water. On the other hand, lead arsenate and soap solution form an objectionable combination because the lead arsenate reacts with the sodium oleate of the soap, forming lead oleate (insoluble in water) and sodium arsenate. The latter is soluble in water and the foliage is injured by the high concentration of soluble arsenic.

Lime-sulphur solution and lead arsenate may be safely mixed because analysis shows that the soluble arsenic is not much greater than when the arsenic compound is shaken with water.

The table shows also that Bordeaux mixture should not be mixed with the group of "emulsified oils" because the emulsion of oils with water containing calcium hydroxide of Bordeaux mixture is not very satisfactory, the result being that some of the unemulsified oil remaining will injure the plant. The reversible nature of soap and oil emulsions in general is discussed by Bancroft[2] as follows:

> "Since sodium oleate emulsifies oil in water and calcium oleate emulsifies water in oil, a mixture of the two oleates will behave differently, depending on the relative amounts. There will also be some ratio of calcium to sodium at which the two oleates will practically balance each other and the slightest relative change will change the type of the emulsion."

The reaction of calcium hydroxide of Bordeaux mixture with sodium oleate of the soap will result in the formation of the maximum quantity of calcium oleate and this will then reverse

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[1] *Cal. Exp. Sta. Circ.*, **195** (1918).

[2] "Applied Colloid Chemistry," p. 267.

TABLE XXVI.—INCOMPATIBILITY OF INSECTICIDES AND FUNGICIDES

Spray compounds	Objectionable combination with:	Questionable combination with:
Stomach Poisons		
Acid lead arsenate	Alkali sulphide Soaps Soap-oil emulsion	Lime-sulphur
Basic lead arsenate		Alkali sulphides
Paris green	Lime-sulphur Alkali sulphides Soaps Soap-oil emulsion Cyanide fumigation	Tobacco infusion
Zinc arsenite	Lime-sulphur Alkali sulphides Soaps Soap-oil emulsion	Bordeaux mixture Cyanide fumigation Tobacco infusion
Tracheal Poisons		
Tobacco infusion	Bordeaux mixture	Paris green Zinc arsenite
Cyanide fumigation	Zinc arsenite Paris green Bordeaux mixture	
Soap-oil emulsion	Zinc arsenite Paris green Acid lead arsenate Lime-sulphur	Bordeaux mixture
Soaps	Zinc arsenite Paris green Acid lead arsenate	Lime-sulphur Bordeaux mixture
Tracheal Poisons and Fungicides		
Alkali sulphides	Zinc arsenite Paris green Acid lead arsenite	Bordeaux mixture Basic lead arsenate
Sulphur		
Lime-sulphur solution	Soap-oil emulsion Zinc arsenate Paris green	Bordeaux mixture Acid lead arsenate Soaps
Fungicides		
Bordeaux mixture	Cyanide fumigation Tobacco infusion	Lime sulphur Alkali sulphides Soaps Soap-oil emulsion Zinc arsenate

Other combinations of these sprays are thought to be safe.

the emulsion to the form of water in oil. The continuous film of oil will then injure foliage with which it comes in contact.

Arsenic Sprays.—Since many insecticidal compounds rely upon arsenic for their effective killing qualities it is important that the student become familiar with a few of the large number of available arsenic compounds. The more important ones are as follows: Arsenic trioxide, As_2O_3 (also called "arsenic" or "white arsenic"); arsenic pentoxide, As_2O_5; arsenic acid, H_3AsO_4; metarsenic acid, $HAsO_3$; potassium arsenite, K_3AsO_3; potassium arsenate, K_3AsO_4; Paris green, $Cu(C_2H_3O_2)_2 \cdot Cu_3(AsO_3)_2$; lead arsenate, $Pb_3(AsO_4)_2$; and calcium arsenate, $Ca_3(AsO_4)_2$.

PARIS GREEN

This is seen from the formula given above to be an aceto-arsenite of copper. Theoretically the compound contains 58.55 per cent of As_2O_3, 31.39 per cent of CuO and 10.06 per cent of $(CH_3CO)_2O$, the respective anhydrides. However, this does not usually represent the actual composition, either of the solid Paris green or of a solution in which it is suspended. Copper arsenate, or even free arsenic or arsenous acids may be present.

Total Arsenic.—The determination of total arsenic in Paris green is based upon the volatility of arsenic trichloride. Cuprous chloride is added to the hydrochloric acid solution in which the sample has been dissolved, and this reduces any pentavalent arsenic to the more volatile, trivalent form. The distillate containing arsenous chloride and hydrochloric acid is absorbed in cold water. The acid is neutralized, sodium bicarbonate is added in excess and the arsenic is titrated with standard iodine solution. The arsenite is oxidized by free iodine as follows:

$$Na_3AsO_3 + I_2 + H_2O \rightarrow Na_3AsO_4 + 2HI.$$

A neutral solution is maintained by the excess of sodium bicarbonate, which neutralizes hydriodic acid as fast as the latter is formed, thus preventing reversal of the reaction.

The apparatus for total arsenic determination is shown in Fig. 61. *A* is a 250-cc distilling flask fitted with a 50-cc dropping funnel; *B*, *C*, and *D* are flasks holding 500, 1000 and 100 cc respectively. *B* and *C* are surrounded by cracked ice and contain 40 and 100 cc of water respectively. The flask *D* contains 50 cc of water, which serves as a trap to prevent entrance of air.

Determination of Total Arsenic: *Distillation Method.*—Prepare the following reagents:

(*a*) *Arsenous Acid.*—Dissolve exactly 2 gm of arsenous oxide of known purity (dried over calcium chloride for ten hours) by boiling with about 200 cc of water containing 10 cc of concentrated sulphuric acid. Cool and transfer to a 500-cc volumetric flask, dilute to the mark and mix thoroughly. Keep stoppered.

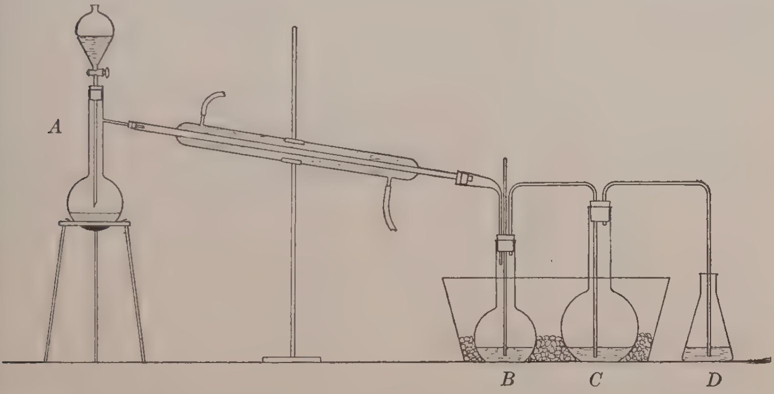

Fig. 61.—Apparatus for the determination of total arsenic by distillation.

(*b*) *Starch Indicator.*—Mix about 0.5 gm of starch with cold water to form a thin paste; add about 100 cc of boiling water and stir thoroughly.

(*c*) *Iodine Solution.*—Dissolve 6.35 gm of iodine and 12.5 gm of potassium iodide in about 100 cc of water, decant from any sediment, dilute to 1000 cc and mix well. Standardize against solution (*a*) as follows:

Using a pipette, measure 50 cc of the arsenous acid solution into an Erlenmeyer flask, dilute to about 400 cc and neutralize with sodium bicarbonate, adding 4 to 5 gm in excess. Add the standard iodine solution from a burette, rotating the flask continuously, until the yellow color disappears only slowly, showing that the end point is near; then add 1 cc of the starch solution and continue adding the iodine solution drop by drop until a permanent blue color is obtained. Calculate the value of the standard iodine solution in terms of arsenous oxide (As_2O_3). Keep the solution stoppered and away from bright light. Even with this precaution the oxidizing value changes and the solution should be standardized within a few hours of the time when it is to be used.

Calculate the theoretical weight of Paris green that would be equivalent to 250 cc of the standard iodine solution. Weigh out this amount and wash it and about 5 gm of cuprous chloride into the 250 distilling flask with 100 cc of concentrated hydrochloric acid. Distill until the volume in the distilling flask is reduced to about 40 cc, then add 50 cc of concentrated hydrochloric acid by means of the dropping funnel. Continue this process of addition of acid and distillation until 200 cc of distillate has been obtained. Wash down the condenser and all connecting tubes, allowing the rinsings to run into the flasks. Transfer the contents of the receiving flasks to a 1000-cc volumetric flask, rinsing the former well, dilute to the mark and mix thoroughly. Measure 100 cc of this solution into a 1000-cc Erlenmeyer flask, add phenolphthalein and nearly neutralize with a concentrated sodium hydroxide solution. The solution should be kept cold. Add 10 gm of sodium bicarbonate and titrate the arsenic with standard iodine solution, using starch as indicator. Calculate the per cent of total arsenic, as both element and arsenous oxide.

The official method for the determination of arsenic, which has just been described, is in some respects less desirable than the method which was formerly official.[1] One of the principal difficulties of the older method is the formation of a yellow colloidal solution of arsenous iodide when potassium iodide and hydrochloric acid are added to reduce the arsenic solution. This color makes impossible the exact removal of iodine by sodium thiosulphate but if the analysis is performed carefully as described below, this difficulty will disappear.

Determination of Total Arsenic and of Copper in Paris Green without Distillation.—To 2 gm of Paris green in a 250-cc flask add about 100 cc of a 2-per cent solution of sodium hydroxide. Boil until all the green compound has been decomposed and only red cuprous oxide remains. Cool, filter into a 250-cc volumetric flask, washing the paper well, dilute to the mark and mix well. Reserve the cuprous oxide on the filter for the copper determination.

Measure two or three portions of 50 cc each of the solution into 250-cc flasks and concentrate by boiling to about half the original volume. Cool to 60°, add 10 cc of concentrated hydrochloric acid and 1 gm of potassium iodide. Mix and allow to stand for 10 minutes. From a burette carefully add sodium thiosulphate solution until the iodine is all reduced. Starch should not be added but care should be exercised in reaching the end point. If a persistent yellow color (see above) develops at this point, use starch solution on a test plate as an outside indicator, touching drops of the titrated solution to the starch. If the end point has been passed, add iodine solution until the iodine-starch reaction is barely produced. Allow to stand for 5 minutes longer and if iodine color reappears carefully add more thiosulphate solution. Immediately add, as rapidly as can be done without loss by effervescence, 15 gm of sodium bicarbonate, free from lumps. Titrate at once with standard iodine solution, deferring the addition of starch until near the end point. Calculate the per cent of total arsenic, expressed as arsenous oxide, in the Paris green.

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[1] *U. S. Dept. of Agr., Chem. Bull.*, **107.**

The residue of cuprous oxide is treated on the filter with 5 cc of nitric acid, specific gravity 1.2, the solution being caught in a 250-cc flask.

Wash the paper well with hot water and proceed as directed for the standardization of sodium thiosulphate against metallic copper, page 162, beginning with "Boil until red fumes have been expelled . . ." Calculate the per cent of copper in the Paris green. The result may also be expressed as cupric oxide, if desired.

Distinction between Arsenates and Arsenites.—It is frequently desirable to distinguish qualitatively between arsenates and arsenites in spray mixtures. Probably the most accurate test for an arsenate depends upon the formation of a precipitate of magnesium ammonium arsenate when a magnesium salt is added to the basic solution:

$$H_3AsO_4 + 3NH_4OH \rightarrow (NH_4)_3AsO_4 + 3H_2O, \qquad (1)$$

$$(NH_4)_3AsO_4 + MgCl_2 \rightarrow MgNH_4AsO_4 + 2NH_4Cl. \qquad (2)$$

Dissolve about 0.5 gm of sodium arsenite and sodium arsenate in separate 10-cc portions of water. Add 3 cc of magnesia mixture to each tube and stir. It will be noted that no precipitate will be produced in the former case but a white crystalline one forms in the latter and adheres to the sides of the vessel. Repeat, using the filtered spray solution instead of known arsenic salts.

Silver nitrate is a reagent which is useful for the detection of arsenites. In a neutral solution of an arsenite this gives a yellow precipitate of silver arsenite while with an arsenate, brown silver arsenate is produced.

Water-soluble Arsenous Oxide.—It has already been stated that water-soluble arsenic (of free arsenous acid or sodium arsenite) is very injurious to young foliage. The Federal insecticide act of 1910 specifies a maximum of 3.5 per cent of water-soluble arsenous oxide in Paris green and not more than 0.75 per cent in lead arsenate paste.

It is very important that the directions as to temperature be observed closely because the amount of soluble arsenic varies considerably with small deviations in temperature.

Determination of Water-soluble Arsenic.—Weigh 2 gm (if a paste, use 4 gm) of the sample on a counterpoised glass or scoop, brush into a 1000-cc volumetric flask, and add nearly 1000 cc of recently boiled distilled water which has been cooled to exactly 32°. Stopper the flask and immerse in a water bath which is kept at 32° (±1°) by means of a thermostat. Digest for 24 hours, shaking hourly for the first eight hours of this period. Dilute to the mark, mix and filter through a dry filter, reject the first 25 cc and collect exactly 250 cc in a volumetric flask. Rinse into a 1000-cc flask or beaker and titrate with the standard iodine solution that was used for total arsenic. Calculate the amount of water-soluble arsenic as arsenous oxide.

LEAD ARSENATE

Of the internal poisons for insects, lead arsenate is used most extensively. Lead arsenate was recommended as an insecticide in 1892 and it was first used against tent caterpillars. It is made by treating disodium arsenate with either lead nitrate or lead acetate. Lead arsenate made from lead nitrate contains about 31.5 per cent of combined arsenic pentoxide while that made from lead acetate contains about 25.5 per cent.

Lead arsenate ("neutral") is gradually replacing Paris green as a spray because of its low degree of solubility, it being a safer spray on this account. The arsenic becomes more soluble if the lead arsenate solution is prepared with water containing sodium sulphate or sodium chloride. Solutions containing only 0.1 per cent of the former or 0.05 per cent of the latter will dissolve an appreciable amount of arsenic from lead arsenate. Spraying tests have shown that 10 grains of sodium chloride per gallon, when used with lead arsenate in the spray fluid, produced injury and 40 grains per gallon injured about half of the foliage. It is therefore important to avoid ordinary mineral water and salt water in preparing the spray.

Lead arsenate has the advantage over Paris green in that it sticks to the foliage well when applied as a spray.

Determination of Moisture: (*a*) *In Powder.*—Weigh a porcelain crucible without cover, then add about 2 gm of sample and reweigh. Dry to constant weight at 105° to 110° and report the loss of weight as moisture.

(*b*) *In Paste.*—In a weighed dish dry 50 gm for one hour at 105° to 110°. Cool and reweigh. Calculate the moisture thus obtained as p. Grind the partly dried sample to a fine powder, mix well and transfer a small portion to a sample bottle. Weigh 2 gm of this into a crucible and dry again for two hours at 105° to 110°. Calculate the loss as per cent, on the basis of the partly dried sample as 100, and call this p'. Total moisture $= M = p + \left(\frac{100 - p}{100}\right)p'$.

Use the anhydrous material for the determination of the total lead oxide and total arsenic.

Determination of Lead Oxide.—Heat on a hot plate about 0.5 gm of the dry powdered sample with about 25 cc of dilute nitric acid (1 to 4) in a 500-cc beaker. If necessary, remove any insoluble residue by filtration. Dilute to about 400 cc and heat nearly to boiling. Add ammonium hydroxide to slight precipitation of basic lead salts, then add dilute nitric acid (1 to 10) to redissolve the precipitate, adding about 2 cc in excess. Pipette into this solution, kept almost at boiling, 50 cc of a hot 10-per cent potassium chromate solution, stirring constantly. Decant while hot through a weighed Gooch filter, previously dried at 150°. Wash several times by decantation and then on the filter paper with boiling water until the washings are colorless. Dry the lead chromate at 140° to 150° to constant weight. Calculate the per cent of lead monoxide in the dried sample. Multiply this per cent by $\frac{100 - p}{100}$ (see determination of moisture) to obtain the per cent based upon the original paste.

Determination of Total Arsenic.—Proceed as directed for the determination of total arsenic in Paris green by the distillation method, page 297. Use about 5 gm of the sample and after the distillate has been diluted to 1000 cc and mixed, measure 100-cc portions for titration. Calculate the per cent of total arsenic, expressed as arsenic pentoxide.

Determination of Total Arsenic Oxide.—Prepare a standard iodine and starch solution as directed in the determination of Paris green on page 297. Prepare also:

Standard Thiosulphate Solution.—An approximately twentieth-normal solution of sodium thiosulphate is prepared by dissolving 13 gm of the crystallized salt in recently boiled and cooled water. Filter and make up to 1000 cc with water treated in the same way. Standardize by the method given on page 162, or by that given on page 178, in either case modifying the treatment to take account of the fact that this solution is only about half as concentrated as the ones described in these references. Calculate the weight of arsenic pentoxide equivalent to 1 cc of the solution.

Weigh accurately about 0.5 gm of the powdered sample, transfer to an Erlenmeyer flask and add 25 cc of concentrated hydrochloric acid. If necessary to effect solution heat on a steam bath, keeping the flask covered in order to prevent evaporation of the acid. Cool to 20°, add 10 cc of 20-per cent potassium iodide solution and 50 cc (more, if necessary to produce a clear solution) of 25-per cent ammonium chloride solution. Immediately titrate the liberated iodine with standard sodium thiosulphate. When the color becomes a faint yellow, dilute with 150 cc of water and continue the titration very slowly, using starch solution near the end point. Calculate the per cent of total arsenic oxide, As_2O_5.

Determination of Water-soluble Arsenic Oxide.—The agents needed are starch indicator, standard arsenous oxide solution and a standard iodine solution. These are prepared as directed on page 297.

To 2 gm of the original sample, if a powder, or 4 gm if a paste, in a 1000-cc volumetric flask, add nearly 1000 cc of recently boiled water which has been cooled to exactly 32°. Stopper the flask and place in a water bath kept at 32° by means of a thermostat. Digest for 24 hours, shaking hourly for eight hours during this period. Dilute to the mark, mix and filter through a dry filter, rejecting the first 25 cc of filtrate. Transfer 250 or 500 cc of the clear filtrate to an Erlenmeyer flask, add 3 cc of concentrated sulphuric acid and evaporate on a hot plate. When the volume reaches about 100 cc add 1 gm of potassium iodide and continue the boiling until the volume is about 40 cc. Cool, dilute to about 200 cc, remove the excess iodine with twentieth-normal sodium thiosulphate, avoiding the use of starch solution, and proceed as directed on page 298 for the determination of arsenic in Paris green, beginning with "nearly neutralize with sodium hydroxide............"

Calculate and report as per cent of water-soluble arsenic oxide, As_2O_5.

Determination of Total Arsenous Oxide.—Prepare the starch indicator, standard arsenous oxide and standard iodine solution as directed in the determination of Paris green on page 297. Prepare also:

(*a*) *Dilute Sulphuric Acid Solution.*—Dilute 15 cc of concentrated sulphuric acid with 85 cc of water.

(*b*) *Sodium Hydroxide Solution.*—Dissolve 25 gm of sodium hydroxide in 50 cc of water.

Weigh 0.25 gm of the powdered sample, transfer to a 200-cc Erlenmeyer flask, add 100 cc of dilute sulphuric acid (*a*), and boil for 30 minutes. Cool, transfer to a 200-cc volumetric flask, dilute to the mark, shake thoroughly and filter through a dry filter. Nearly neutralize 100 cc of the filtrate with sodium hydroxide (*b*), using a few drops of phenolphthalein as indicator. If the neutral point is passed, make acid again with dilute sulphuric acid. Continue as directed in the determination of total arsenic in Paris green, page 299, beginning with the neutralization by sodium bicarbonate. Calculate the per cent of total arsenous oxide in the sample.

CALCIUM ARSENATE

This is one of the newer insecticides. It is somewhat similar to arsenate of lead but, in its present form, it is not recommended for use on the more sensitive foliage, such as that of the stone fruits, because of the large amount of water-soluble arsenic it often contains, this causing considerable damage to foliage.

Two of the arsenates of calcium are quite stable: tricalcium arsenate, $Ca_3(AsO_4)_2$ and dicalcium arsenate, $CaHAsO_4$. The tricalcium arsenate may be made in two ways, as follows:

$$3CaHAsO_4 + 2NaOH \rightarrow Ca_3(AsO_4)_2 + Na_2HAsO_4 + 2H_2O; \quad (1)$$
$$2H_3AsO_4 + 3Ca(OH)_2 \rightarrow Ca_3(AsO_4)_2 + 6H_2O. \quad (2)$$

Dicalcium arsenate dissolves in water to the extent of 0.33 gm in 100 cc at 25° while tricalcium arsenate is soluble to the extent of only 0.014 gm at the same temperature. The solubility of the first salt is so large that there is danger of damage when it is applied to tender foliage. Also, unless it has been prepared with care, it may contain quantities of the easily soluble disodium arsenate, as shown in Eq. (1). This has been largely overcome by adding an excess of lime water, which reacts with any dicalcium arsenate or disodium arsenate to form the less soluble tricalcium salt.[1] The powdered calcium arsenates on the market contain approximately 52 per cent of arsenic, calculated as pentoxide, while the paste contains less, according to the proportion of water retained.

Determination of Total Arsenic.—Proceed by the distillation method as with Paris green, using 2 to 2.5 gm of sample. Calculate as the pentoxide.

LIME-SULPHUR SOLUTION

This spray is important in the control of San Jose and other scales. It is effective also in the extermination of numerous insects. This is especially true when it is combined with lead arsenate and nicotine and it is used then for the simultaneous destruction of many sucking and chewing insects and of fungus diseases. The standard lime-sulphur solution consists of calcium tetrasulphide, pentasulphide and thiosulphate in a water solution. It is produced by boiling lime water containing sulphur. The probable reactions are generally understood to be as follows:

$$3Ca(OH)_2 + 10S \rightarrow 2CaS_4 + CaS_2O_3 + 3H_2O. \quad (1)$$

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[1] See also REEDY and HAAG, *J. Ind. Eng. Chem.*, **13**, 1038 (1921).

The calcium thiosulphate thus formed is largely decomposed by boiling, calcium sulphite and free sulphur being formed:

$$CaS_2O_3 \rightarrow CaSO_3 + S. \qquad (2)$$

The free sulphur formed in reaction (2) is dissolved by calcium tetrasulphide to form pentasulphide.

$$CaS_4 + S \rightarrow CaS_5. \qquad (3)$$

The insoluble sludge remaining consists of a mixture of calcium sulphite and some calcium sulphate, the latter being formed by oxidation of sulphite.

Extensive investigations on the fungicidal value of sulphur of polysulphides were carried on by Syre, Solmon and Warmall,[1] using the hop-mildew at its most resistant stage as their standard. They have expressed the opinion that the fungicidal value depends upon the percentage of polysulphide sulphur in solution, rather than the total sulphur content.

Lime-sulphur solutions, either upon standing exposed to air or after being sprayed, slowly react with oxygen, forming calcium thiosulphate and free sulphur:

$$2CaS_5 + 3O_2 \rightarrow 2CaS_2O_3 + 3S_2$$

Determination of Total Sulphur.—Weigh a closed weighing bottle then add about 10 cc of the lime-sulphur solution, close and weigh again. Rinse into a 250-cc volumetric flask and dilute to the mark with recently boiled and cooled distilled water and mix thoroughly. Dissolve 2 to 3 gm of sodium peroxide in 50 cc of cold distilled water in a 250-cc beaker. Pipette 10 cc of the prepared lime-sulphur solution to this solution, keeping the tip of the pipette just under the surface of the solution until it is to be raised for drainage at the end of the process. Cover immediately with a watch glass and warm on a steam bath with frequent shakings until the sulphur is oxidized to sulphate (the yellow color having disappeared), adding more sodium peroxide if necessary. Dilute to 25 cc, acidify with hydrochloric acid, evaporate to dryness, treat with 25 cc of water acidified with 5 cc of hydrochloric acid, boil and filter to remove silica if present. Dilute the filtrate to about 200 cc and heat to boiling. Add a drop of methyl red then neutralize with sulphur-free ammonium hydroxide. Add 1 cc of approximately normal (1 to 10) hydrochloric acid, then add 10 to 25 cc (as found to be necessary) of 10-per cent barium chloride solution, slowly from a pipette, stirring constantly. Digest on a steam bath until the precipitate settles readily, then filter through quantitative filter paper. Wash until free from chlorides and burn the paper in an inclined weighed crucible at a low temperature (not above dull redness). When the precipitate is white, cool and weigh. Calculate the sulphur from the weight of barium sulphate. Corrections should be made for any sulphur present in the reagents, determined by a blank experiment. Sodium peroxide, especially, is liable to contain sulphates.

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[1] *J. Agr. Sci.*, **9**, 283 (1919).

Determination of Total Sulphide Sulphur.—Dissolve 50 gm of zinc chloride in about 500 cc of water and add 125 cc of concentrated ammonium hydroxide, which will redissolve the precipitate first formed. Add 50 gm of ammonium chloride and dilute to about 1 liter.

Pipette 10 cc of the lime-sulphur solution (freshly made as for the total sulphur determination) into a 250-cc beaker, dilute to 100 cc and add ammoniacal zinc solution until the sulphide sulphur is all precipitated, as indicated by the failure of a drop of the clear solution to darken a few drops of dilute nickel sulphate solution. Filter immediately, wash the precipitate thoroughly with cold water and return it and the filter to the beaker. Cover with water, disintegrate with a glass rod and slowly add about 3 gm of sodium peroxide, keeping the beaker well covered with the watch glass. Warm on the steam bath, with frequent shaking, until all of the sulphur is oxidized to sulphate and the precipitate is all dissolved, adding more sodium peroxide if necessary. Make slightly acid with hydrochloric acid, filter to remove shreds of filter paper, wash thoroughly with hot water, heat the filtrate and washings to boiling and determine the sulphur as described for total sulphur, neutralizing and acidifying in the same manner. Calculate the per cent of sulphide sulphur in the sample.

Total Calcium.—The per cent of calcium in a lime-sulphur solution will depend upon the character and purity of the lime used in its preparation, as well as upon dilution and degree of hydrolysis. It will vary over wide limits but as this element is of relatively small importance in connection with insecticidal properties, its determination is not often required. The following method is official:

Determination of Total Calcium.—To 25 cc of the lime-sulphur solution, prepared as for the preceding determination, add 10 cc of concentrated hydrochloric acid and evaporate to dryness on the steam bath. Add 25 cc of water and 5 cc of concentrated hydrochloric acid, warm until all of the calcium chloride is dissolved and filter from sulphur and any silica that may be present. Make slightly ammoniacal, boil and filter from iron and aluminium hydroxides if these are produced. Heat to boiling and precipitate the calcium with ammonium oxalate solution and finish the determination as described on page 64 or 69, Part I. Calculate the per cent of calcium oxide in the sample.

NICOTINE INSECTICIDES

Nicotine in solution is an effective agent for destroying many soft bodied insects, as aphides and pear psyllæ. Solutions of nicotine are valuable as insecticides because of the intensely poisonous character of nicotine, whether eaten by the insect or absorbed through its exterior covering. They may be applied in various dilutions and in combinations with other sprays to treat, all at once, certain sucking and biting insects and fungus parasites. Nicotine is not injurious to foliage, in ordinary concentrations. As a vegetable alkaloid it is a weak base and this makes it possible to determine the amount of nicotine present in a solution by titrating with a standard acid.

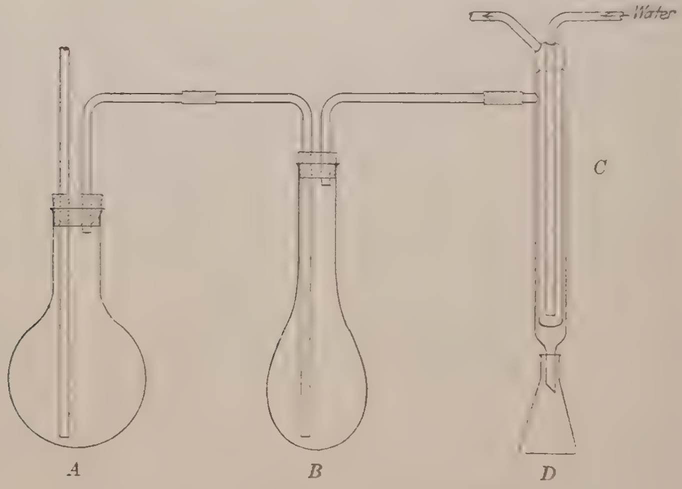

FIG. 62.—Apparatus for distillation with steam.

Most dry tobacco waste contains from 2 to 3 per cent of nicotine. An extract may be prepared for use as an insecticide by stirring 25 to 30 lb. of the tobacco waste with 50 gal. of water. This will make a solution averaging about 0.06 per cent of nicotine.[1]

The separation of nicotine from a solution is made by extracting with ether. The extracted residue is dissolved in a base solution and the nicotine separated by steam distillation. The nicotine in the distillate is titrated with a standard acid as follows:

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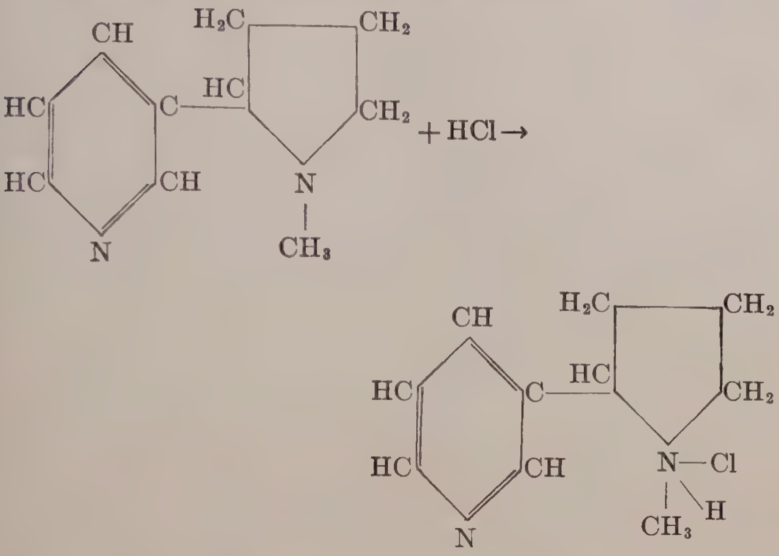
[1] *Va. Exp. Sta. Bull.*, **208** (1914).



Determination of Nicotine.—Prepare the following solutions:

(*a*) *Alcoholic Sodium Hydroxide Solution.*—Dissolve 6 gm of sodium hydroxide in 40 cc of water and 60 cc of 90-per cent alcohol.

(*b*) *Approximately tenth-normal sodium hydroxide solution*, not standardized.

(*c*) *Tenth-normal sulphuric acid*, accurately standardized against pure sodium carbonate (see pages 58 *et seq*, Part I).

Weigh into a 50-cc beaker, 5 to 6 gm of tobacco extract or 20 gm of finely powdered tobacco or tobacco waste which has been dried at 60°. Add 10 cc of alcoholic sodium hydroxide and, in the case of tobacco extract, follow with enough shredded filter paper to form a moist but not lumpy mass. Mix thoroughly, transfer to a continuous extractor (page 146) and extract for about five hours with ether. Evaporate the ether at a low temperature and take up the residue with 50 cc of sodium hydroxide (*b*). Transfer the residue by means of 200 cc of water to a 500-cc Kjeldahl flask, add a piece of pumice or a small amount of crushed porcelain and a small piece of paraffine, heat to boiling and distill by steam, passing the distillate through a condenser cooled by a rapidly flowing current of water. Distill from 400 to 500 cc, stopping the current of steam and using a flame under the flask at a point such that only about 15 cc of the liquid finally remains in the flask. Titrate the distillate with tenth-normal sulphuric acid, using methyl red as an indicator. Calculate the per cent of nicotine in the sample.

BORDEAUX MIXTURE

Bordeaux mixture consists of copper sulphate and calcium hydroxide. It is one of the most reliable of the fungicides, its poisonous properties being due to the copper and hydroxyl ions. Chemical tests show that when Bordeaux mixture is applied to the leaf, a small amount of copper enters and combines with chlorophyl of the cells. This seems to give the leaf an increased resistance to insect injury. The spray spreads rapidly over the leaf and forms a thin colloidal membrane composed of basic copper and calcium salts. Both copper and calcium hydroxide are fungicidal and when spores fall upon a sprayed leaf, they are either killed or germinate very slowly.

Moisture.—The determination of moisture in Bordeaux mixture powder is made by drying at 105° to 110° to constant weight. The determination in the paste is complicated by the fact that basic carbonates of copper (formed through interaction of copper sulphate, calcium hydroxide and carbonic acid) lose carbon dioxide during the first drying process:

$$(CuOH)_2CO_3 \rightarrow 2CuO + CO_2 + H_2O.$$

A determination of carbon dioxide must then be made and the proper correction applied.

Determination of Moisture: (*a*) *In Powder.*—Dry 2 gm of sample as directed for lead arsenate powder, page 300. Calculate the loss as moisture.

(*b*) *In Paste.*—Heat about 100 gm (weighed in a porcelain dish) at 90° to 100° until dry enough to powder readily. Weigh and calculate the per cent loss. Denote this by a.

Powder the partly dried sample, mix and determine the per cent loss on drying about 2 gm of this as directed above for powder. Call this b. Determine carbon dioxide (see below) in both paste and partly dried powder. Let c = per cent of carbon dioxide in the partly dried material and d the total carbon dioxide in the paste. Since b and c are based upon a partly dried sample the factor $\frac{100 - a}{100}$ will correct these to a basis of the original paste. Then total moisture

$$M = a + \left(\frac{100 - a}{100}\right)(b + c) - d.$$

(The student should prove this formula. Note that the formula given in the Official Methods, separate volume, first edition, is incorrect.)

Determination of Carbon Dioxide.—Weigh 2 gm of the powder or 10 gm of the paste, place in the reaction flask together with 20 cc of water and determine the carbon dioxide by one of the methods discussed on pages 77 to 85, Part I. Calculate the per cent of carbon dioxide in the sample as used.

Determination of Copper.—Prepare solutions as directed for the determination of copper in cuprous oxide, page 162 (feeds). Weigh about 2 gm of the sample, dissolve in about 50 cc of 10-per cent nitric acid and add ammonium hydroxide solution in slight excess. Then without removing the precipitate which has formed, add acetic acid to clear and 5 to 10 cc in excess. Cool, add 10 cc of 30-per cent potassium iodide solution and titrate with thiosulphate as directed on page 162. Calculate the per cent of copper present in the sample (dried, partly dried or paste) and in the sample as received.

SOAP SPRAYS AND EMULSIONS

Soaps are used to a considerable extent for making oil emulsions and they are often added to other sprays to cause the latter to spread uniformly and to adhere more closely to the foliage. The soap-kerosene emulsions are used somewhat for the soft-bodied sucking insects, such as aphides, but they are being replaced, by solutions of nicotine sulphate. Soap-oil emulsions are used for scale insects.

Determination of Moisture in Soap.—Weigh rapidly about 5 gm of the carefully selected sample into a weighed 50-cc beaker in which has been placed a one-half inch layer of recently ignited dry sand and a small glass rod. If the soap is hard, cut it up into very thin strips. Add 25 cc of alcohol (more if necessary) and dissolve the soap by warming on a steam bath, stirring constantly. Evaporate the alcohol, heat in an oven at 110°, stirring occasionally, until the soap is nearly dry, then weigh; dry again for 30 minutes and weigh. Continue this process until the weight changes only a few milligrams during 30 minutes of drying.

CHLORPICRIN

Chlorpicrin is trichlornitromethane, CCl_3NO_2. It is rated as 283 times as toxic as carbon disulphide, compared on a basis of molecular weights. It is not as inflammable as is carbon disulphide, and its vapor is about twice as heavy, which feature makes it quite desirable for grain fumigation. Chlorpicrin vapor is so very poisonous[1] and active that not more than one-half pound is needed for the fumigation of 1000 cu. ft. of space. Ten times this amount of carbon disulphide would be required.

Much work is being done upon the adaptation of other poison gases to insecticidal and fungicidal uses. No doubt this field will be developed very rapidly during the next few years and the agricultural analyst will have many new problems presented for his solution, as a result.

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[1] *J. Econ. Ent.*, **11**, 4 (1918).

APPENDIX

LOGARITHMS

Natural Numbers	0	1	2	3	4	5	6	7	8	9	Proportional Parts								
											1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4	8	12	17	21	25	29	33	37
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	3	6	8	11	14	17	20	22	25
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	5	7	8	9	10
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9
46	6628	6637	6646	6656	6665	6675	6384	6693	6702	6712	1	2	3	4	5	6	7	7	8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	5	6	7	8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	4	5	6	7	8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	4	5	6	7	8
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	3	4	5	6	7	8
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	3	4	5	6	7	8
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7

Logarithms

Natural Numbers	0	1	2	3	4	5	6	7	8	9	Proportional Parts								
											1	2	3	4	5	6	7	8	9
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7
57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6
69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	3	4

ANTILOGARITHMS

Logarithms	0	1	2	3	4	5	6	7	8	9	Proportional Parts								
											1	2	3	4	5	6	7	8	9
.00	1000	1002	1005	1007	1009	1012	1014	1016	1019	1021	0	0	1	1	1	1	2	2	2
.01	1023	1026	1028	1030	1033	1035	1038	1040	1042	1045	0	0	1	1	1	1	2	2	2
.02	1047	1050	1052	1054	1057	1059	1062	1064	1067	1069	0	0	1	1	1	1	2	2	2
.03	1072	1074	1076	1079	1081	1084	1086	1089	1091	1094	0	0	1	1	1	1	2	2	2
.04	1096	1099	1102	1104	1107	1109	1112	1114	1117	1119	0	1	1	1	1	2	2	2	2
.05	1122	1125	1127	1130	1132	1135	1138	1140	1143	1146	0	1	1	1	1	2	2	2	2
.06	1148	1151	1153	1156	1159	1161	1164	1167	1169	1172	0	1	1	1	1	2	2	2	2
.07	1175	1178	1180	1183	1186	1189	1191	1194	1197	1199	0	1	1	1	1	2	2	2	2
.08	1202	1205	1208	1211	1213	1216	1219	1222	1225	1227	0	1	1	1	1	2	2	2	3
.09	1230	1233	1236	1239	1242	1245	1247	1250	1253	1256	0	1	1	1	1	2	2	2	3
.10	1259	1262	1265	1268	1271	1274	1276	1279	1282	1285	0	1	1	1	1	2	2	2	3
.11	1288	1291	1294	1297	1300	1303	1306	1309	1312	1315	0	1	1	1	2	2	2	2	3
.12	1318	1321	1324	1327	1330	1334	1337	1340	1343	1346	0	1	1	1	2	2	2	2	3
.13	1349	1352	1355	1358	1361	1365	1368	1371	1374	1377	0	1	1	1	2	2	2	3	3
.14	1380	1384	1387	1390	1393	1396	1400	1403	1406	1409	0	1	1	1	2	2	2	3	3
.15	1413	1416	1419	1422	1426	1429	1432	1435	1439	1442	0	1	1	1	2	2	2	3	3
.16	1445	1449	1452	1455	1459	1462	1466	1469	1472	1476	0	1	1	1	2	2	2	3	3
.17	1479	1483	1486	1489	1493	1496	1500	1503	1507	1510	0	1	1	1	2	2	2	3	3
.18	1514	1517	1521	1524	1528	1531	1535	1538	1542	1545	0	1	1	1	2	2	2	3	3
.19	1549	1552	1556	1560	1563	1567	1570	1574	1578	1581	0	1	1	1	2	2	3	3	3
.20	1585	1589	1592	1596	1600	1603	1607	1611	1614	1618	0	1	1	1	2	2	3	3	3
.21	1622	1626	1629	1633	1637	1641	1644	1648	1652	1656	0	1	1	2	2	2	3	3	3
.22	1660	1663	1667	1671	1675	1679	1683	1687	1690	1694	0	1	1	2	2	2	3	3	3
.23	1698	1702	1706	1710	1714	1718	1722	1726	1730	1734	0	1	1	2	2	2	3	3	4
.24	1738	1742	1746	1750	1754	1758	1762	1766	1770	1774	0	1	1	2	2	2	3	3	4
.25	1778	1782	1786	1791	1795	1799	1803	1807	1811	1816	0	1	1	2	2	2	3	3	4
.26	1820	1824	1828	1832	1837	1841	1845	1849	1854	1858	0	1	1	2	2	3	3	3	4
.27	1862	1866	1871	1875	1879	1884	1888	1892	1897	1901	0	1	1	2	2	3	3	3	4
.28	1905	1910	1914	1919	1923	1928	1932	1936	1941	1945	0	1	1	2	2	3	3	4	4
.29	1950	1954	1959	1963	1968	1972	1977	1982	1986	1991	0	1	1	2	2	3	3	4	4
.30	1995	2000	2004	2009	2014	2018	2023	2028	2032	2037	0	1	1	2	2	3	3	4	4
.31	2042	2046	2051	2056	2061	2065	2070	2075	2080	2084	0	1	1	2	2	3	3	4	4
.32	2089	2094	2099	2104	2109	2113	2118	2123	2128	2133	0	1	1	2	2	3	3	4	4
.33	2138	2143	2148	2153	2158	2163	2168	2173	2178	2183	0	1	1	2	2	3	3	4	4
.34	2188	2193	2198	2203	2208	2213	2218	2223	2228	2234	1	1	2	2	3	3	4	4	5
.35	2239	2244	2249	2254	2259	2265	2270	2275	2280	2286	1	1	2	2	3	3	4	4	5
.36	2291	2296	2301	2307	2312	2317	2323	2328	2333	2339	1	1	2	2	3	3	4	4	5
.37	2344	2350	2355	2360	2366	2371	2377	2382	2388	2393	1	1	2	2	3	3	4	4	5
.38	2399	2404	2410	2415	2421	2427	2432	2438	2443	2449	1	1	2	2	3	3	4	4	5
.39	2455	2460	2466	2472	2477	2483	2489	2495	2500	2506	1	1	2	2	3	3	4	5	5
.40	2512	2518	2523	2529	2535	2541	2547	2553	2559	2564	1	1	2	2	3	4	4	5	5
.41	2570	2576	2582	2588	2594	2600	2606	2612	2618	2624	1	1	2	2	3	4	4	5	5
.42	2630	2636	2642	2649	2655	2661	2667	2673	2679	2685	1	1	2	2	3	4	4	5	6
.43	2692	2698	2704	2710	2716	2723	2729	2735	2742	2748	1	1	2	3	3	4	4	5	6
.44	2754	2761	2767	2773	2780	2786	2793	2799	2805	2812	1	1	2	3	3	4	4	5	6
.45	2818	2825	2831	2838	2844	2851	2858	2864	2871	2877	1	1	2	3	3	4	5	5	6
.46	2884	2891	2897	2904	2911	2917	2924	2931	2938	2944	1	1	2	3	3	4	5	5	6
.47	2951	2958	2965	2972	2979	2985	2992	2999	3006	3013	1	1	2	3	3	4	5	5	6
.48	3020	3027	3034	3041	3048	3055	3062	3069	3076	3083	1	1	2	3	4	4	5	6	6
.49	3090	3097	3105	3112	3119	3126	3133	3141	3148	3155	1	1	2	3	4	4	5	6	6

ANTILOGARITHMS

Logarithms	0	1	2	3	4	5	6	7	8	9	Proportional Parts								
											1	2	3	4	5	6	7	8	9
.50	3162	3170	3177	3184	3192	3199	3206	3214	3221	3228	1	1	2	3	4	4	5	6	7
.51	3236	3243	3251	3258	3266	3273	3281	3289	3296	3304	1	2	2	3	4	5	5	6	7
.52	3311	3319	3327	3334	3342	3350	3357	3365	3373	3381	1	2	2	3	4	5	5	6	7
.53	3388	3396	3404	3412	3420	3428	3436	3443	3451	3459	1	2	2	3	4	5	6	6	7
.54	3467	3475	3483	3491	3499	3508	3516	3524	3532	3540	1	2	2	3	4	5	6	6	7
.55	3548	3556	3565	3573	3581	3589	3597	3606	3614	3622	1	2	2	3	4	5	6	7	7
.56	3631	3639	3648	3656	3664	3673	3681	3690	3698	3707	1	2	3	3	4	5	6	7	8
.57	3715	3724	3733	3741	3750	3758	3767	3776	3784	3793	1	2	3	3	4	5	6	7	8
.58	3802	3811	3819	3828	3837	3846	3855	3864	3873	3882	1	2	3	4	4	5	6	7	8
.59	3890	3899	3908	3917	3926	3936	3945	3954	3963	3972	1	2	3	4	5	5	6	7	8
.60	3981	3990	3999	4009	4018	4027	4036	4046	4055	4064	1	2	3	4	5	6	6	7	8
.61	4074	4083	4093	4102	4111	4121	4130	4140	4150	4159	1	2	3	4	5	6	7	8	9
.62	4169	4178	4188	4198	4207	4217	4227	4236	4246	4256	1	2	3	4	5	6	7	8	9
.63	4266	4276	4285	4295	4305	4315	4325	4335	4345	4355	1	2	3	4	5	6	7	8	9
.64	4365	4375	4385	4395	4406	4416	4426	4436	4446	4457	1	2	3	4	5	6	7	8	9
.65	4467	4477	4487	4498	4508	4519	4529	4539	4550	4560	1	2	3	4	5	6	7	8	9
.66	4571	4581	4592	4603	4613	4624	4634	4645	4656	4667	1	2	3	4	5	6	7	9	10
.67	4677	4688	4699	4710	4721	4732	4742	4753	4764	4775	1	2	3	4	5	7	8	9	10
.68	4786	4797	4808	4819	4831	4842	4853	4864	4875	4887	1	2	3	4	6	7	8	9	10
.69	4898	4909	4920	4932	4943	4955	4966	4977	4989	5000	1	2	3	5	6	7	8	9	10
.70	5012	5023	5035	5047	5058	5070	5082	5093	5105	5117	1	2	4	5	6	7	8	9	11
.71	5129	5140	5152	5164	5176	5188	5200	5212	5224	5236	1	2	4	5	6	7	8	10	11
.72	5248	5260	5272	5284	5297	5309	5321	5333	5346	5358	1	2	4	5	6	8	9	10	11
.73	5370	5383	5395	5408	5420	5433	5445	5458	5470	5483	1	3	4	5	6	8	9	10	11
74	5495	5508	5521	5534	5546	5559	5572	5585	5598	5610	1	3	4	5	6	8	9	10	12
.75	5623	5636	5649	5662	5675	5689	5702	5715	5728	5741	1	3	4	5	7	8	9	10	12
.76	5754	5768	5781	5794	5808	5821	5834	5848	5861	5875	1	3	4	5	7	8	9	11	12
.77	5888	5902	5916	5929	5943	5957	5970	5984	5998	6012	1	3	4	5	7	8	10	11	12
.78	6026	6039	6053	6067	6081	6095	6109	6124	6138	6152	1	3	4	6	7	8	10	11	13
.79	6166	6180	6194	6209	6223	6237	6252	6266	6281	6295	1	3	4	6	7	9	10	11	13
.80	6310	6324	6339	6353	6368	6383	6397	6412	6427	6442	1	3	4	6	7	9	10	12	13
.81	6457	6471	6486	6501	6516	6531	6546	6561	6577	6592	2	3	5	6	8	9	11	12	14
.82	6607	6622	6637	6653	6668	6683	6699	6714	6730	6745	2	3	5	6	8	9	11	13	14
.83	6761	6776	6792	6808	6823	6839	6855	6871	6887	6902	2	3	5	6	8	9	11	13	14
.84	6918	6934	6950	6966	6982	6998	7015	7031	7047	7063	2	3	5	6	8	10	11	13	15
.85	7079	7096	7112	7129	7145	7161	7178	7194	7211	7228	2	3	5	7	8	10	12	13	15
.86	7244	7261	7278	7295	7311	7328	7345	7362	7379	7396	2	3	5	7	8	10	12	13	15
.87	7413	7430	7447	7464	7482	7499	7516	7534	7551	7568	2	3	5	7	9	10	12	14	16
.88	7586	7603	7621	7638	7656	7674	7691	7709	7727	7745	2	4	5	7	9	11	12	14	16
.89	7762	7780	7798	7816	7834	7852	7870	7889	7907	7925	2	4	5	7	9	11	13	14	16
.90	7943	7962	7980	7998	8017	8035	8054	8072	8091	8110	2	4	6	7	9	11	13	15	17
.91	8128	8147	8166	8185	8204	8222	8241	8260	8279	8299	2	4	6	8	9	11	13	15	17
.92	8318	8337	8356	8375	8395	8414	8433	8453	8472	8492	2	4	6	8	10	12	14	15	17
.93	8511	8531	8551	8570	8590	8610	8630	8650	8670	8690	2	4	6	8	10	12	14	16	18
.94	8710	8730	8750	8770	8790	8810	8831	8851	8872	8892	2	4	6	8	10	12	14	16	18
.95	8913	8933	8954	8974	8995	9016	9036	9057	9078	9099	2	4	6	8	10	12	15	17	19
.96	9120	9141	9162	9183	9204	9226	9247	9268	9290	9311	2	4	6	8	11	13	15	17	19
.97	9333	9354	9376	9397	9419	9441	9462	9484	9506	9528	2	4	7	9	11	13	15	17	20
.98	9550	9572	9594	9616	9638	9661	9683	9705	9727	9750	2	4	7	9	11	13	16	18	20
.99	9772	9795	9817	9840	9863	9886	9908	9931	9954	9977	2	5	7	9	11	14	16	18	20

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INTERNATIONAL ATOMIC WEIGHTS

	Symbol	Atomic weight		Symbol	Atomic weight
Aluminum	Al	26.97	Mercury	Hg	200.61
Antimony	Sb	121.77	Molybdenum	Mo	96.0
Argon	A	39.91	Neodymium	Nd	144.27
Arsenic	As	74.96	Neon	Ne	20.2
Barium	Ba	137.37	Nickel	Ni	58.69
Beryllium	Be	9.02	Nitrogen	N	14.008
Bismuth	Bi	209.0	Osmium	Os	190.8
Boron	B	10.82	Oxygen	O	16.00
Bromine	Br	79.916	Palladium	Pd	106.7
Cadmium	Cd	112.41	Phosphorus	P	31.027
Calcium	Ca	40.07	Platinum	Pt	195.23
Carbon	C	12.00	Potassium	K	39.096
Cerium	Ce	140.25	Praseodymium	Pr	140.92
Cesium	Cs	132.81	Radon	Rn	222.0
Chlorine	Cl	35.457	Radium	Ra	225.95
Chromium	Cr	52.01	Rhodium	Rh	102.91
Cobalt	Co	58.94	Rubidium	Rb	85.44
Columbium	Cb	93.1	Ruthenium	Ru	101.7
Copper	Cu	63.57	Samarium	Sa	150.43
Dysprosium	Dy	162.52	Scandium	Sc	45.1
Erbium	Er	167.7	Selenium	Se	79.2
Europium	Eu	152.0	Silicon	Si	28.06
Fluorine	F	19.0	Silver	Ag	107.88
Gadolinium	Gd	157.3	Sodium	Na	22.997
Gallium	Ga	69.72	Strontium	Sr	87.63
Germanium	Ge	72.6	Sulfur	S	32.065
Glucinum	Gl	9.02	Tantalum	Ta	181.5
Gold	Au	197.2	Tellurium	Te	127.5
Helium	He	4.00	Terbium	Tb	159.2
Holmium	Ho	163.4	Thallium	Tl	204.39
Hydrogen	H	1.008	Thorium	Th	232.15
Hafnium	Hf	178.6	Thulium	Tm	169.4
Indium	In	114.8	Tin	Sn	118.7
Iodine	I	126.932	Titanium	Ti	48.1
Iridium	Ir	193.1	Tungsten	W	184.0
Iron	Fe	55.84	Uranium	U	238.17
Krypton	Kr	82.9	Vanadium	V	50.96
Lanthanum	La	138.9	Xenon	X	130.2
Lead	Pb	207.2	Ytterbium	Yb	173.6
Lithium	Li	6.94	Yttrium	Y	88.9
Lutecium	Lu	175.0	Zinc	Zn	65.38
Magnesium	Mg	24.32	Zirconium	Zr	91.
Manganese	Mn	54.93			